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PITUITARY AND CARBOHYDRATE METABOLISM OF THE BRAIN

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CHANGES in the brain function of certain patients are often connected with obvious disturbances of the pituitary function. In rats, changes of the brain function occur regularly after hypophysectomy. In order to analyse these changes, investigations of hexokinase activity and anaerobic glycolysis of the grey matter of the brain have been carried out.

METHODS

The grey matter was carefully removed with bent iris scissors from the brain, and put into ice-chilled distilled water, homogenised, then pipetted into tubes which contained: 0.25 ml. A.T.P. (150 mg/10 ml), 0.06 ml. $MgCl_2$ (M/6), 0.09 ml. NaF (M), 0.45 ml. veronal buffer (pH 7.6), 0.15 ml. glucose solution (10 mg/ml) and a control tube with distilled water instead of glucose.

After thorough mixing of the contents of the tubes, samples were taken for initial sugar estimation. The tubes were then incubated for 30 minutes at 30°C. after which time further samples were taken for analysis. The Hagedorn-Jensen sugar method was used with the modification that the proteins were precipitated with barium hydroxide and zinc sulphate. The amount of sugar which had disappeared was calculated per mg N_2 of the homogenate.

The anaerobic glycolysis was determined in tissue slices of the grey matter, the determinations being carried out in accordance with the Warburg method in Warburg Ringer solution containing 0.2% glucose.

The oxygen consumption of the tissue slices was determined with the direct Warburg method in conical flasks containing Ringer solution with sugar.

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Only male Wistar rats weighing 120 to 150 grs. were used for the investigation. They were killed by decapitation. Hypophysectomy was carried out by the paratracheal method.

RESULTS

There is a considerable increase in the hexokinase activity of the grey matter of the brain 10 to 12 days after hypophysectomy, the increase varying between 37 and 140% (Table I). Brains investigated 5 to 7 days after hypophysectomy (not included in Table I) showed an increase of at least 25%.

TABLE I. HEXOKINASE ACTIVITY OF BRAIN (GREY MATTER) OF 150–170 G. MALE NORMAL RATS AND RATS HYPOPHYSECTOMIZED 10–20 DAYS BEFORE INVESTIGATION

Experiment No.	Grey matter hexokinase activity per mg. N_2 in 30 minutes—glucose equivalent		Changes
	Normal controls	Hypophysectomized animals	
1	0.32	0.46	+ 43%
2	0.38	0.58	+ 52%
3	0.28	0.50	+ 78%
4	0.41	0.72	+ 75%
5	0.27	0.65	+140%
6	0.28	0.49	+ 75%
7	0.36	0.50	+ 39%
8	0.35	0.48	+ 37%

Two to 5 days after bilateral adrenalectomy, the increase in the hexokinase activity is equally outspoken, as can be seen from Table II.

TABLE II. HEXOKINASE ACTIVITY OF BRAIN (GREY MATTER) OF 120–150 G. MALE NORMAL RATS AND RATS ADRENALECTOMIZED 2 TO 5 DAYS BEFORE INVESTIGATION

Experiment No.	Grey matter hexokinase activity per mg. N_2 in 30 minutes—glucose equivalent		Changes
	Normal controls	Adrenalectomized animals	
1	0.36	0.49	+ 36%
2	0.24	0.48	+100%
3	0.31	0.67	+116%

The anaerobic glycolysis of tissue slices of the grey matter three weeks after hypophysectomy was found increased by 30% on the average (Table III). Treatment with corticotrophic hormone reduced it again to a normal level (Table III).

The oxygen consumption of the brain showed hardly any change three weeks after hypophysectomy (Table III). This was in contrast to the behaviour of other tissues of the animals where decrease of the oxygen consumption was considerable.

TABLE III

No. of Rat	Treatment	Brain (grey matter) metabolism	
		$QO_2 \pm \sigma$	$Q_G^{N_2} \pm \sigma$
11	Normal controls	7.1 ±0.8	20.4 ±1.3
16	Hypophysectomized 3 weeks ago	7.8 ±0.9	26.2 ±1.6
14	Hypophysectomized 3 weeks ago, treated with 80 units corticotrophic hormone (total dose) for one week	7.6 ±1.1	20.9 ±1.7

DISCUSSION

The increased hexokinase activity of brains of hypophysectomised and adrenalectomised rats was initially regarded as a somewhat unexpected result since generally a reduction of the different functions is taking place after hypophysectomy. It may be assumed that some inhibitory substance which is produced in the adrenal cortex or in the pituitary has been removed by the operation. This substance or these substances may be related to the substances recently described by Colowick, Cori and Slein (1947) who in *in vitro* experiments found inhibition of the hexokinase activity after addition of adrenal cortex extracts and pituitary anterior lobe fractions. They claimed that highly purified preparations of adrenocorticotrophic hormone do not produce this inhibition. Nevertheless, study of the procedure described by Cori and her coworkers for the preparation of the inhibitory substance makes it obvious that all her extracts contained adrenocorticotrophic hormone—it remains to be seen whether as the active substance or as an unessential byproduct. Taking into account the multitude of active substances produced by the adrenal cortex the existence of several active adreno-corticotrophic principles ought to be considered. The active substance decisive for the hexokinase inhibition need not necessarily have been present in the highly purified preparation of adreno-corticotrophic hormone at the disposal of Cori and coworkers. In our own experiments where the anaerobic glycolysis was found significantly increased after hypophysectomy, adrenocorticotrophic hormone fractions (prepared by isoelectric precipitation at pH 4.7)inhibited the increase of brain glycolysis in vivo.

Surveying the above results it can be concluded that, after hypophysectomy, a decisive disturbance develops in the carbohydrate household of the brain. The brain consumes and metabolises an increased amount of sugar. Consequently, an exhaustion of sugar reserves takes place which might easily account for fits seen occasionally in hypophysectomised but otherwise untreated animals before their spontaneous death. Apparently, the brain burns more sugar after hypophysectomy. This wastage seems to be characteristic for the

brain of hypophysectomised animals, and may very well be the cause of other pathological changes occurring after hypophysectomy.

SUMMARY

The hexokinase activity of suspensions made from the grey matter of the brain was found increased after hypophysectomy and adrenalectomy.

The anaerobic glycolysis of slices of the grey matter of the brain was found increased after hypophysectomy, and could be reduced by treatment with adreno-corticotrophic hormone.

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TISSUE CHANGES AND SODIUM BALANCE IN PANTOTHENIC ACID-DEFICIENT RATS

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LIPOID DEPLETION, or hemorrhage and necrosis of the adrenal cortex, has been recorded by numerous investigators of pantothenic acid deficiency (Daft and Sebrell, 1939; Nelson, 1939; Ashburn, 1940; Mills *et al.*, 1940; Morgan and Simms, 1940 (a); Salmon and Engel, 1940; Mushett and Unna, 1941; Supplee *et al.*, 1942 and Deane and McKibbin, 1946). Ashburn (1940) suggested that these morphological changes may be accompanied by varying degrees of adrenal cortical insufficiency. The occurrence in deficient animals of sudden prostration, gastro-intestinal symptoms (Shaeffer *et al.*, 1942) and a terminal picture consistent with severe dehydration would seem to support this concept. Also consistent with this premise is the decreased resistance to water intoxication in pantothenic acid deficient animals observed by Gaunt *et al.*, 1946. Furthermore, pantothenic acid deficient rats have been observed to consume larger quantities of salt when on a self-selection salt intake than do similarly treated controls (Supplee *et al.*, 1942).

Accordingly, it seemed desirable to study adrenal cortical function as related to salt and water metabolism in pantothenic acid deficiency. The development of the flame photometer (Barnes *et al.*, 1945) made it feasible to study sodium balance in an adequate sample of pantothenic acid deficient rats and to attempt to correlate physiologic findings with the morphologic changes demonstrable by the use of certain histochemical techniques (Dempsey and Wislocki, 1946).

Because of reported thymic atrophy in pantothenic acid deficiency (Deane and McKibbin, 1946; Ashburn, 1947) and the current interest in the relationship between adrenal cortical activity and the involution of lymphoid tissues (Dougherty and White, 1945) a comparative study of these organs seemed indicated.

METHODS

The metabolism experiments involved a total of 42 weanling Sprague-Dawley rats. At the beginning of the experiment these varied in age from 21 to 24 days and in weight from 30 to 45 grams. They were divided into 7 groups of 6 rats each. Three groups were fed a pantothenic acid deficient

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diet (#1872), 3 groups were used as controls (diet 1871) and one group was placed on a diet deficient in riboflavin (diet 1873). This latter group was used to check on the specificity of the finding in pantothenic acid deficient animals. The control groups of animals were as follows: (1) pair-fed controls—pair-fed with their litter mates in one of the pantothenic deficient groups; (2) subacute inanition controls—receiving control diet *ad lib.* until 8 days before termination of the experiment when their food intake was restricted to two grams per rat per day; (3) *ad lib.* controls. The rats were equally divided according to sex in the untreated pantothenic acid deficient and pair-fed control groups. Only male rats were used in the other groups.

The basic control diet 1871 had the following composition: vitamin-free casein (Labco) 18%, "sodium free" salt mixture[1] (Orent-Keiles *et al.*, 1937) 4%, riboflavin 400 mgm. distributed in 100 gm. of anhydrous dextrose 0.5%, anhydrous dextrose (Merck) 69.5%, Crisco 8.0%. Vitamins were incorporated in the diet at the following levels per 10 gm. of diet: thiamine 100 gamma, pyridoxine 100 gamma, choline 20 mgm., pantothenic acid 200 gamma, folic acid 40 gamma, nicotinic acid 500 gamma, biotin 0.5 gamma, inositol 4.0 mgm. In addition, 2000 units of vitamin A and 400 units of vitamin D were given biweekly and 3 mg. of alpha tocopherol once weekly. The two additional diets used, diet 1872, pantothenic acid deficient, and diet 1873, riboflavin deficient, differed from the basic diet only in the omission of those specific vitamins. During the development of the deficiency and prior to the metabolism periods, to be later described all animals received *ad lib.* amounts of 0.145% sodium chloride in distilled water; this dilute saline solution was offered in graduated drinking tubes and the individual intakes recorded daily.

After 32 days on their respective experimental diets all animals in the above groups were placed in individual metabolism cages for a five day period. For the first three days of the metabolism period salt was withdrawn by substituting distilled water for the usual dilute saline drinking water. This procedure sharply curtailed sodium intake. Following the three day depletion period the animals entered a two day recovery phase during which dilute saline (0.145% NaCl) was again offered as drinking water. Accurate records of food and fluid intake were continued throughout the metabolism period and total urine samples were recovered at 24 hour intervals for each rat.

At the beginning of the metabolism period, one pantothenic acid deficient group was treated with pantothenic acid, a second group received desoxycorticosterone acetate and a third group remained untreated. The desoxycorticosterone acetate (Roche Organon[2]) was given subcutaneously in peanut oil in a dose of 1.5 mgm./rat/day throughout the 5 day metabolism period. The pantothenic acid treated group received calcium pantothenate in aqueous solution subcutaneously in a dose of 1 mgm. per rat/day for a similar period.

By utilizing the flame photometer (Barnes, 1945) according to the procedure outlined by Hald (1946) it was possible to carry out sodium and potassium determinations on the 210 urine samples obtained with consider-

[1] On analysis of the diet the sodium content was found to be 0.025 m.eq. per gram. The salt mixture contributed 0.184 m.eq. of potassium per gram of diet.

[2] The desoxycorticosterone acetate was generously supplied by Dr. K. W. Thompson, Medical Director, Roche Organon, Nutley, N. J.

able facility and reasonable accuracy. Preliminary experiments with this type of metabolism setup indicated that the error in urinary sodium and potassium determinations by this method ranged from 5 to 10%. During the actual determinations on the urine samples the error was usually less than 5%. Triplicate determinations were made on each sample. It was felt that such results would be sufficiently accurate to detect the gross urinary alterations characteristic of frank adrenal cortical insufficiency (Rubin and Krick, 1936).

At the conclusion of each metabolism period all animals were weighed and chloroformed. Complete autopsies were then performed, and tissues from all organs were placed in buffered neutral formalin for subsequent histologic study. The spleen, adrenals, thymuses and cervical lymph nodes of each animal were stripped clean and weighed to the nearest milligram on a torsion balance. The central nervous system was not examined. Azure-eosinate and Van Gieson stains were carried out routinely on all tissues. Frozen sections of liver, kidney and adrenals were stained for fat using the supersaturated isopropanol Sudan technique of Lillie and Ashburn (1943). In addition, the following procedures were carried out on frozen sections of the adrenals: (1) Fixed sections cut at 35 micra were stained with 2,4-dinitrophenylhydrazine according to the technique of Albert and Leblond (1946) modified by the substitution of isopropanol for ethanol. Similar sections previously treated with acetone and alcohol were likewise stained with 2,4-dinitrophenylhydrazine and used as control sections. (2) Frozen sections cut at 15 micra were studied unstained for birefringent material.

RESULTS

The weight curves of pair-fed and deficient animals were virtually identical through the third week. A downward trend in the weight curves of deficient animals appeared between the 25th and the 32nd day so that at the termination of the experiment (at 38 days) pair-fed controls averaged 58 gms. while their deficient litter mates averaged 47 gms. Preceding or coincident with their failure to gain, the deficient animals developed dry, rough, lack-lustre coats and many showed porphyrin incrustation about their nostrils.

During the 8 day terminal period of food restriction, animals in the subacute inanition group showed an average weight loss of 30 gms. Final mean weight was, for this group, 104 gms. The final mean weight of the *ad lib.* control rats was 155 gms.

Electrolyte balance. Sodium balance studies carried out on a total of 42 animals failed to reveal any defect in sodium conservation in pantothenic acid deficient animals when compared with control groups. Deficient animals maintained a positive daily sodium balance in spite of a sharply curtailed sodium intake during a three-day depletion period. During the subsequent two-day recovery period when 0.145% NaCl was offered *ad lib.*, deficient animals retained relatively large amounts of sodium (0.3 to 0.4 m.eq./Na/rat/day) showing no significant difference from pair-fed desoxycorticosterone acetate-treated and pantothenic acid-treated controls, cf. table 1. Potassium balance studies were likewise noncontributory.

TABLE 1. SODIUM BALANCE M.EQ.—Na/24 HOURS
Mean values—(6 rats per group)

Group	Sodium restriction period*						Recovery period, 0.145% salt solution *ad lib.*					
	2nd 24 hours			3rd 24 hours			1st 24 hours			2nd 24 hours		
	Intake	Output	Bal.	Intake	Output	Bal.	Intake	Output	Bal.	Intake	Output	Bal.
Pantothenic Acid Deficiency	.061	.028	+.033	.065	.023	+.042	.378	.042	+.336	.265	.083	+.182
Pair-fed (control)	.074	.039	+.035	.053	.023	+.030	.381	.037	+.344	.401	.067	+.334
Pantothenic Acid Deficiency; Treated with DOCA**	.063	.027	+.036	.046	.014	+.032	.639	.255	+.384	No data		—
Pantothenic Acid Deficiency; Treated with Pantothenic Acid	.052	.056	−.004	.052	.020	+.032	.270	.146	+.124	.285	.110	+.175
Ad Lib. (control)	.145	.013	+.132	.166	.011	+.155	1.276	.306	+.970	.789	.288	+.501
Subacute Inanition (control)	.030	.100	−.070	.030	.020	+.010	.496	.211	+.285	.370	.196	+.174
Riboflavin Deficiency	.059	.016	+.043	.050	.008	+.042	.631	.262	+.369	.456	.201	+.255

* Sodium content of diet—0.025 m.eq. per gram.
** DOCA = desoxycorticosterone acetate.

During the fourth and fifth weeks after being placed on their respective diets the mean daily, per capita, *ad lib.* fluid (0.145% NaCl) intake of pantothenic acid deficient, pair-fed control and riboflavin deficient animals were compared over a ten-day period. Comparison of the values for the five groups reveals no statistically significant differences in the saline intakes which ranged from 7.4 to 9.3 cc. of 0.145% NaCl solution per rat per day.

Comparative organ weights. All organ weights (table 2) are expressed in milligrams per 100 gm. body weight and each value is accompanied by its respective standard error.

Pantothenic acid deficient animals show significantly lower thymic weights (54.8 ± 15.6) than pair-fed controls (171.1 ± 22.9). However, *ad lib.* controls show relatively high thymic weights (277.6 ± 17.3), emphasizing the importance of inanition in the production of both preceding values. Thus pantothenic acid deficiency appears to accentuate the thymic involution of inanition, but the effect is apparently nonspecific, for riboflavin deficient litter mates likewise show comparably low thymic weights (68.5 ± 9.7). The low thymic weights of deficient animals are uninfluenced by the administration of DOCA. Following treatment with pantothenic acid the mean thymic weight is 141.7 or 86.9 mg. greater than in untreated rats. However, in view of the small number of animals in the groups this difference is not considered significant.

TABLE 2. MEAN ORGAN WEIGHTS WITH STANDARD ERROR
(expressed in mg. per 100 gm. of body weight)

Rat Groups	No. of animals	Thymus		Lymph nodes		Spleen		Adrenals	
		mean wt.	S.E.	mean wt.	S.E.	mean wt.	S.E.	mean wt.	S.E.
Pantothenic acid deficiency	6	54.8	15.6	112.9	17.3	219.4	21.0	35.0	3.6
Pair-fed (control)	6	171.1	22.9	170.2	12.4	236.7	3.2	39.2	1.8
Pantothenic acid deficiency Rx with DOCA*	4	63.1	15.2	95.6	10.7	193.8	22.7	32.8	2.4
Pantothenic acid deficiency Rx with PA**	4	141.7	32.5	120.2	21.6	230.9	7.8	33.6	2.5
Ad lib. (control)	6	277.6	17.3	118.6	16.5	257.5	14.0	26.4	2.4
Subacute inanition (control)	6	108.1	17.4	101.7	10.5	174.6	19.4	32.3	3.0
Riboflavin deficiency	6	68.5	9.7	101.8	12.2	229.6	7.3	46.0	2.0

* DOCA = Desoxycorticosterone acetate.
** PA = Pantothenic acid.

Pantothenic acid deficient animals show no statistically significant differences in splenic and adrenal weights when compared with their pair-fed controls.

When compared with *ad lib.* controls both the pair-fed and riboflavin deficient rats showed enlarged adrenals. The adrenals of pantothenic acid-deficient rats, however, were not enlarged when similarly compared.

The data regarding lymph node weights are not sufficiently clear cut to permit definitive conclusions. Lymph node weights show a wide

range of variation, with those of the pair-fed group of animals significantly higher (170.2 ± 12.4) than all other groups.

Adrenals. The adrenals of pantothenic acid-deficient animals when compared with pair-fed controls show a moderately severe cortical lipoid depletion, which spares the glomerulosa and sharply delimits it from the remaining cortex. This type of adrenal lipoid distribution was not observed in riboflavin deficient animals, *ad lib.* controls, or in that group of controls subjected to a period of subacute inanition, but has been previously recorded in pantothenic acid deficiency (Ashburn, 1940; Deane and McKibbin, 1946).

The glomerulosa of deficient animals contains normal amounts of compactly disposed lipoid droplets. The fasciculata, in contrast, shows moderate to marked lipoid depletion of its outer half with subtotal to complete depletion of its inner third. Not infrequently the entire fasciculata shows subtotal lipoid depletion. The lipoid free zone of the outer fasciculata is widened in all instances where its inner limits are demonstrable.

The distribution of acetone-alcohol soluble phenylhydrazine-positive material in the adrenals of pantothenic acid deficient rats is essentially the same as that of the sudanophilic lipoid. The glomerulosa is again sharply demarcated in deficient animals and stains an intense light yellow. The fasciculata shows a weak pale yellow reaction in its outer half, grading into a negative reaction in its inner third.

In unstained preparations, the quantity of birefringent material is likewise markedly reduced or absent in the fasciculata of deficient animals, while abundant in the corresponding zone of pair-fed controls. From the standpoint of more exact localization, however, birefringent material does not completely parallel sudanophilia or the phenylhydrazine reaction. In the outer fasciculata of deficient animals where small quantities of sudanophilic and phenylhydrazine-positive material are often demonstrable, birefringence is not infrequently completely absent. This lack of parallelism is even more striking in the glomerulosa where there is marked variation in the quantity of birefringent material in both control and deficient groups without corresponding variation in the phenylhydrazine reaction or the degree of sudanophilia.

In addition to these alterations in lipoid content, phenylhydrazine staining and birefringence, the adrenals of 2 in 12 pantothenic acid deficient animals show the necrotizing hemorrhagic cortical lesions often noted in association with this deficiency.

As previously reported (Ashburn 1940), the adrenals of pantothenic acid-deficient rats show a prompt and striking, although occasionally incomplete restoration of lipoid when treated for five days with pantothenic acid. This restoration of sudanophilic lipoid following pantothenic acid treatment is accompanied by a parallel increase in the intensity of the phenylhydrazine reaction and a return of bire-

fringence to virtually normal levels. The significance of these observations is increased by the fact that while under treatment, the individual daily food intake of animals was arbitrarily restricted to its former low pretreatment level (3.5 gm. diet/rat/day).

Thymus. In all but one of the untreated pantothenic acid deficient group of animals the thymus is small showing moderate to marked atrophy with subtotal loss of lymphoid tissue and persistence of the reticuloendothelial and epithelial elements. Similar, though less marked histological changes, are noted in riboflavin deficient animals, but are not observed in pair-fed control animals suffering from a comparable degree of chronic inanition.

In the pair-fed, *ad lib.*, and subacute inanition controls, the thymuses show well defined cortico-medullary differentiation with well preserved lymphoid tissue.

This relatively normal picture is likewise noted in pantothenic acid deficient animals receiving five days' treatment with pantothenic acid. Persistent thymic atrophy was observed in two pantothenic acid deficient animals, dying after only three days' treatment with pantothenic acid.

Lymph nodes. Moderate lymphoid atrophy is observed in cervical lymph nodes of pantothenic acid deficient animals when compared with their pair-fed controls. A diffuse process, the atrophy is characterized by an over-all reduction in lymph node size and a disappearance or shrinkage or primary follicles, often giving the cortico-medullary lymphoid pulp an abnormally loose-textured appearance.

This degree of lymphoid atrophy is not observed in lymph nodes of pantothenic acid deficient animals after five days' treatment with pantothenic acid. Following such treatment follicles are prominent and active. Lymph nodes of riboflavin deficient animals and those of the *ad lib.* and subacute inanition groups likewise show only minor changes. In contrast, the nodes of desoxycorticosterone acetate treated animals show lymphoid atrophy similar to that seen in untreated pantothenic acid deficient animals.

Bone and Marrow. In all but two animals of the untreated pantothenic acid deficient series, the femoral and tibial epiphyseal plates show evidence of reduced cartilaginous and osteoblastic activity as previously reported (by Ashburn, 1940). Similar, though less marked, changes are noted in the riboflavin deficient series.

This degree of suppression of bony and cartilaginous activity is not observed in pair-fed control animals suffering from a comparable degree of chronic inanition and growth retardation.

Pair-fed animals and the *ad lib.* and subacute inanition control groups show a wide epiphyseal plate with orderly proliferation and degeneration of cartilage and active osteogenesis.

Following treatment with desoxycorticosterone acetate the histologic appearance of the epiphyses in deficient animals is apparently

unaffected, showing the same suppression of cellular activity observed in untreated animals. In all animals receiving five days' treatment with pantothenic acid, however, the epiphyses appear normally active resembling those of the various control series.

The bone marrow of untreated pantothenic acid deficient animals appears normal or hyperplastic and, except for a left shift in granulocytes, its cellular composition shows no consistent deviations from the normal as detectable by the study of marrow sections. Following treatment with pantothenic acid the left shift in marrow granulocytes was no longer evident and in some animals metamyelocytes and segmented forms appeared to be present in greater than normal numbers.

DISCUSSION

Fouts *et al.* (1940) studying blood electrolytes in filtrate factor II deficiency in dogs found no evidence suggesting adrenal insufficiency and Wintrobe (1943) noted evidence of adequate sodium conservation in several pantothenic acid deficient pigs. In agreement with these findings is the normal behavior of pantothenic acid deficient rats with respect to sodium balance and conservation under the conditions of the present study.

The increased sensitivity to the water test (Gaunt *et al.*, 1946) and the increased salt consumption (Supplee, 1942) of pantothenic acid deficient rats are not adequately explained by our data. It should be pointed out that in our experiments the rats did not have the opportunity of making a selection between salt and salt-free water as was true in Supplee's study. With reference to Gaunt's results, it is possible that the water test might detect a mild adrenal insufficiency not demonstrable by sodium balance studies.

It has been suggested by Deane and McKibbin (1946) that the cortical lipoid depletion and involution of lymphoid tissues as observed in pantothenic acid deficient rats are nonspecific alterations which can be duplicated by a wide variety of "alarming stimuli" (Deanesly, 1931; Selye, 1937; Dalton *et al.*, 1944; Popjak, 1944; Sayers *et al.*, 1944). The demonstration that hypersecretion of pituitary adrenotropin and corticosterone actually exists under such conditions must await the application of improved bioassay methods, but some interesting speculations regarding this possibility have been offered (Deane and McKibbin, 1946).

On the basis of observed morphological differences, several authors (Ashburn, 1940; Swann, 1940; Sarason, 1943) have suggested that the different layers of the adrenal cortex may have quite independent functions. Expanding this concept, others (Dalton et *al.*, 1944; Deane and Greep, 1946) inferred that the glomerulosa produces desoxycorticosterone-like hormones which affect electrolyte balance, while the fasciculata produces corticosterone-like hormones which are related to the process of gluconeogenesis. The preservation of a normal

glomerulosal layer (Ashburn, 1940; Deane and McKibbin, 1946) in pantothenic acid deficient animals associated with an apparently intact sodium metabolism is consistent with this thesis. We are in agreement with Deane and McKibbin (1946) that lipoid depletion of the fasciculata can occur under a variety of conditions and in this sense is a nonspecific reaction.

The thymic atrophy of pantothenic acid deficient rats (Deane and McKibbin 1946) and its apparent response to pantothenic acid treatment (Ashburn 1947) have been previously recorded, but its mechanism remains obscure. Of interest, however, is the absence of comparable atrophy in pair-fed controls and the fact that the gland apparently responds to pantothenic acid treatment while food intake remains constant.

The thymic involution observed cannot, however, be regarded as specific for pantothenic acid deficiency, for similar though less marked atrophic changes were noted in riboflavin deficient animals.

SUMMARY

In sodium metabolism studies involving 42 Sprague-Dawley rats, pantothenic acid deficient animals on drastically restricted sodium intakes show no defect in sodium conservation, demonstrable by flame photometry. Various prominent histopathologic changes in the adrenals, thymus, lymph nodes and long bones are also recorded.

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STUDIES ON THE BIOASSAY OF HORMONES: THE RELATIVE POTENCIES OF TESTOSTERONE PROPIONATE AND TESTOSTERONE-17-β-DIETHYL AMINO ETHYL CARBONATE HYDROCHLORIDE[1]

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THIS STUDY is concerned with the relative biological activity of a water soluble derivative of testosterone, testosterone-17-β-diethyl amino ethyl carbonate hydrochloride (T.W.S.), and testosterone propionate (T.P.). The interest in the water soluble derivative was prompted by a desire to employ the compound for in vitro studies on the metabolism of the androgens as well as the influence of the compound on enzyme systems of specific tissues.

EXPERIMENTAL

The comparative studies were done on the comb of the White Leghorn chick. The chicks were obtained from Kerr Chickeries, Frenchtown, New Jersey. The animals were received in Cleveland on the second or third day after hatching and the experiments started three days after hatching. The material was dissolved in water or peanut oil depending on the particular experiment. The steroids[2] were administered for seven successive days starting with the third day after hatching. In some experiments the material was applied directly on the comb. Under these conditions the total dose of steroid was dissolved in 0.35 cc. of solution and 0.05 cc. was administered daily from a hyperdermic syringe fitted with a number 24 needle. In other experiments the material was injected subcutaneously and the daily dose was contained in 0.70 cc., 0.10 cc. being administered daily.

The chicks were autopsied 24 hours after the last injection at which time the comb weights and the body weights were determined. Since less variation has been observed in the ratio of the comb weight to the

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[2] The testosterone propionate and testosterone-17-β-diethyl amino ethyl carbonate hydrochloride were generously supplied by Ciba Pharmaceutical Products, Inc.

body weight than in the comb weight alone all statistical evaluations were done on the basis of the comb ratio.

Regression coefficients were calculated by the method of Fisher (1934). The relative potencies were assessed by the method of Irwin (1937) and the error of the potency ratios was calculated by the method of Bülbring (1938).

The relative potencies of the testosterone propionate and the water soluble derivative were determined under the following conditions:

A. When the propionate and the water soluble derivative as the hydrochloride were administered in oil solution by direct application to the chick's comb,
B. When the water soluble derivative was applied to the comb in oil as the free amine and compared to the propionate applied in oil solution,
C. When both the propionate and the water soluble derivative were contained in oil solution and injected subcutaneously,
D. When both derivatives of testosterone were injected subcutaneously, the propionate in oil solution and the water soluble derivative in water.

Table 1 summarizes the results of the responses of the chick comb to the two androgens under various experimental conditions. The various dosage levels at which the individual comparisons were made are tabulated along with the regression coefficients which were calculated from the original data using the relationship of the logarithm of the dose and the response of the comb expressed as the ratio of the comb weight in milligrams to the body weight in grams. The total number of animals used for any one comparison and the sex of the animals are also indicated. For each comparison the significancy of the difference between the slopes of the linear relationships was calculated and found not to be statistically different.

Table 2 is a summary of the relative potencies that were found under the various experimental conditions studied, that is under the four experimental conditions that were outlined previously (A through D). In addition to the relative potencies the error range of the potency ratio has also been calculated. In all calculations the factor of differences in the molecular weight of the compounds has been taken into account and the potency ratios have therefore been adjusted to molar potency ratios.

The highest potency for the water soluble compound in relation to that found for the propionate was under the conditions described by the classification A when both of the compounds were dissolved in oil and applied directly to the surface of the chick's comb. Under these experimental conditions the water soluble derivative was found to possess an activity equivalent to 62 per cent of that of testosterone

TABLE 1. REGRESSION COEFFICIENTS UNDER VARIOUS EXPERIMENTAL CONDITIONS

Experimental Conditions	Testosterone Propionate μg.	T.W.S. μg.	Total number of animals and sex	Slope b	Constant a
A. Both applied to comb in oil solution	10, 20, 40		150 F	0.33	0.66
		40, 80, 160		0.45	0.75
B. Both applied to comb in oil solution TWS as free amine	40, 80, 160		116 M	0.48	0.70
		100, 200, 400		0.46	0.69
C. Both injected subcutaneously in oil solution	50 80, 160		63 F	0.27	0.48
		100, 200, 400		0.44	0.42
	40, 80, 160		122 F	0.71	0.70
		40, 80, 160, 320		0.51	0.55
	40, 80, 160		149 M	0.27	0.47
		100, 200, 400		0.25	0.41
	40, 80, 160		133 M	0.48	0.70
		40, 80, 160, 320		0.26	0.56
D. Both injected subcutaneously T.P. in oil T.W.S. in water	40, 80, 160		76 M	0.35	0.57
		565, 800, 1600		0.30	0.55
	40, 80, 160		72 M	0.34	0.54
		565, 800, 1600		0.29	0.58

propionate. The error of this determination was found to be −44 to +77 per cent.

When the free amine was studied in place of the hydrochloride and compared to testosterone propionate the free amine was found to possess 39 per cent of the activity of the propionate. These conditions are referred to under heading B.

Under the conditions designated as C, the potency of the water soluble derivative was 40 per cent of testosterone propionate. Four comparisons were carried out, two with male chicks and two with female chicks. The potency ratios ranged from 29 to 50 per cent of testosterone propionate.

A great difference in potency ratio was found when the conditions labeled as D were fulfilled. Under these conditions the water soluble compound was administered subcutaneously in water solution while the testosterone propionate was administered in peanut oil. Under these conditions the water soluble compound showed a low degree of

biological activity. The testosterone propionate in oil proved to be about eight times more active than the water soluble compound in water. In the two experiments 11 per cent was found using the female chicks and 14 per cent using the male chicks.

TABLE 2. THE POTENCY OF TESTOSTERONE-17-β-DIETHYL AMINO ETHYL CARBONATE HYDROCHLORIDE (T.W.S.) IN TERMS OF TESTOSTERONE PROPIONATE (T.P.) UNDER VARIOUS EXPERIMENTAL CONDITIONS

Conditions	Potency of T.W.S. in terms of T.P. (Mol basis) %		Error range of potency ratio (P = 0.95) %	
	M	F	M	F
A. Both applied to comb in oil solution	—	62	—	−44; +46
B. Both applied to comb in oil solution T.W.S. as free amine	39	—	−31; +45	—
C. Both injected subcutaneously in oil solution	29	50	−34; +52	−24; +32
	33	49	−33; +50	+49; +98
D. Both injected subcutaneously T.P. in oil T.W.S. in water	14	11	−50; +102	−47; +90

DISCUSSION

In an earlier study by Miescher, Kägi, Scholz, Wettstein and Tschopp (1937) testosterone-17-β-diethyl amino ethyl carbonate hydrochloride was found to possess about 20 per cent the activity of testosterone propionate on the basis of weight. The test consisted in the subcutaneous injection of the material in oil to capons and determining the comb stimulation. The results of our comparative studies of the relative activities of T.W.S. and T.P. when the materials were administered subcutaneously agree well with those of Miescher *et al.* (1937) when both sets of data are considered on a molar basis.

When injected in aqueous solution the T.W.S. showed a comparatively low biological activity. This low biological activity may be due to the relatively quick absorption, inactivation and excretion.

SUMMARY

The comparative androgenic activity of testosterone-17-β-diethyl amino ethyl carbonate hydrochloride (T.W.S.) and testosterone propionate (T.P.) was studied using the chick's comb as the end point. The relative activities have been expressed on a molar basis.

When both androgens were applied to the chick's comb in oil solution the T.W.S. was found to be 62 per cent as active as T.P. with an error range at P = 0.95 of −44 to +77 per cent. If both androgens

were applied to the comb but the free amine was used instead of the hydrochloride of T.W.S., the latter steroid was found to be 39 per cent as active as T.P. The error range at $P = 0.95$ was -31 to $+45$ per cent. When both steroids were injected subcutaneously the T.W.S. was found to be 40 per cent as active as T.P. Finally, when the T.W.S. was injected subcutaneously in aqueous solution and compared to T. P. injected in oil the latter androgen was found to be only 12.5 per cent as active as the T.P.

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STUDIES ON THE BIOASSAY OF HORMONES: THE ASSAY OF CHORIONIC GONADOTROPHIN FROM HUMAN PREGNANCY URINE AND SERUM[1]

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IN A PREVIOUS STUDY we assayed the concentrations of chorionic gonadotrophin in human pregnancy urine by the response of the uterus of the immature rat (Rubin, Dorfman, and Miller, 1946). The method has been studied further in some detail using a different experimental design. Previously we determined the regression line of the international standard of pregnancy urine gonadotrophin by relating the logarithm of the dose to the response (expressed as the ratio of the uterus in milligrams to body weight in grams) and referred the response of the unknown to the standard curve. One striking disadvantage of such a method is the possibility of some change in the variables of the assay from the time of the determination of the standard curve to the time at which the unknown responses are determined. A second disadvantage arises since no check on the qualitative response can be made. Since only one point on the curve is employed it is impossible to determine whether the dose response curve of the unknown is parallel to that of the standard. The present experimental design prevents such errors from occurring since the standard is run in parallel with the unknown at each assay and the significancy of difference of the slopes is easily tested.

ANIMALS, METHODS, MATERIALS

Sprague-Dawley albino rats were employed throughout the study. The age range was from 21 to 24 days and the weights of the animals varied from 35 to 50 grams at the time of the first injection. The total dose of chorionic gonadotrophin was administered subcutaneously in six doses. Two doses daily of 1 cc. each, at least four hours apart, were administered for three consecutive days. On the fifth day (approximately 96 hours after the first injection) the animals were sacrificed at which time uterine weight and body weight were determined. All responses are expressed as the ratio of the uterine weight in milligrams to the body weight in grams.

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The statistical method has been described previously by Bliss (1944) who applied this design to the assay of penicillin. Essentially the design consists in employing four groups of animals, two groups on the standard and two on the unknown. For the purpose of this study we have employed the international standard of urinary hormone for both the reference standard and the unknown. For the serum gonadotrophin a single serum was used as the standard and unknown for all the studies. The concentrations of the standard and the unknown are so chosen that the ratio of the high dose to that of the low dose is the same for both the unknown and the standard as expressed in equation A.

$$\text{A.} \qquad \frac{\text{High Dose of Standard}}{\text{Low Dose of Standard}} = \frac{\text{High Dose of Unknown}}{\text{Low Dose of Unknown}}$$

With this experimental design the calculations of relative potency, the mean slope (b), the standard deviation (s), the error range, and the significance of difference of the respective slopes (t) may be easily calculated. These calculations are carefully illustrated by Bliss (1944) and need not be further elaborated here.

RESULTS

Urinary Chorionic Gonadotrophin (Human Pregnancy)

In order to test the efficiency of the method with respect to urinary chorionic gonadotrophin a series of comparisons were made as illustrated in tables 1, 2, and 3 where the standard was in reality compared to itself or the standard was equal to the unknown. Since previous experience has showed that a straight line relation for the logarithm of the dose versus response held between total doses of 0.25 I.U. and 1.00 I.U. all comparisons were made within this dosage range. The results in Table 1 illustrate comparisons made when the ratio of the high dose to the low dose was 1.5. The designation N in this and other tables indicates the number of sets of animals employed,

TABLE 1. ASSAY OF CHORIONIC GONADOTROPHIN FROM URINE BY UTERINE WEIGHT (RAT)

$$\frac{\text{High Dose (Standard)}}{\text{Low Dose (Standard)}} = \frac{\text{High Dose (Unknown)}}{\text{Low Dose (Unknown)}} = 1.5$$

Standard = Unknown

N	Conc. I.U.	b	s	λ	t	Potency ratio ±S. E. %	Error range P=0.95 %
5	0.5; 0.75	2.71 3.65	0.529 0.547	0.195 0.150	1.335 0.665	90 ± 14 79 ± 16	−27; +38 −34; +52
5	0.4; 0.6	5.80	0.619	0.107	0.541	107 ± 12	−20; +25
5	0.25; 0.37	2.59	0.370	0.143	1.255	94 ± 14	+25; +34
7	0.25; 0.37	2.05	0.318	0.155	1.301	109 ± 15	−24; +32
7	0.5; 0.75	2.73	0.568	0.208	1.400	79 ± 16	−34; +52

TABLE 2. ASSAY OF CHORIONIC GONADOTROPHIN FROM URINE BY UTERINE WEIGHT (RAT)

$$\frac{\text{High Dose (Standard)}}{\text{Low Dose (Standard)}} = \frac{\text{High Dose (Unknown)}}{\text{Low Dose (Unknown)}} = 2$$

Standard = Unknown

N	Conc. I.U.	b	s	λ	t	Potency ratio ±S. E. %	Error range $P = 0.95$ %
5	0.4; 0.8	2.03	0.297	0.146	1.231	100 ± 15	−24; +32
5	0.5; 1.0	2.89 4.55	0.517 0.500	0.179 0.110	0.701 0.168	70 ± 20 93 ± 11	+34; +51 −20; +26
5	0.37; 0.75	3.60 4.78	0.654 0.467	0.182 0.098	0.875 1.799	113 ± 22 96 ± 10	−32; +46 −18; +22
5	0.25; 0.50	3.55 2.86	0.300 0.555	0.085 0.194	1.845 0.555	79 ± 7.3 108 ± 15	−17; +20 −25; +33
7	0.4; 0.8	1.80	0.322	0.179	0.159	94 ± 15	−26; +37
7	0.5; 1.0	3.48	0.536	0.154	0.482	70 ± 15	−26; +37
7	0.37; 0.75	3.73	0.589	0.158	0.550	109 ± 15	−24; +32
10	0.5; 1.0	3.72	0.515	0.138	0.322	83 ± 8.6	−19; +24
10	0.37; 0.75	4.19	0.537	0.128	1.074	103 ± 9.6	−17; +21
10	0.25; 0.50	3.14	0.385	0.123	1.362	90 ± 8.3	−17; +21

TABLE 3. ASSAY OF CHORIONIC GONADOTROPHIN FROM URINE BY UTERINE WEIGHT (RAT)

$$\frac{\text{High Dose (Standard)}}{\text{Low Dose (Standard)}} = \frac{\text{High Dose (Unknown)}}{\text{Low Dose (Unknown)}} = 3$$

Standard = Unknown

N	Conc. I.U.	b	s	λ	t	Potency ratio +S. E. %	Error range $P = 0.95$ %
5	0.25; 0.75	3.14 3.15	0.481 0.478	0.153 0.152	0.450 0.304	98 ± 15 97 ± 15	+27; +37 +27; +57
5*	0.37; 1.00	3.38 5.00	0.532 0.984	0.157 0.197	1.257 0.504	100 ± 16 98 ± 20	−28; +38 −34; +54
7	0.25; 0.75	3.24	0.448	0.138	1.086	96 ± 12	−21; +27
10	0.25; 0.75	3.14	0.452	0.144	0.563	96 ± 10	−19; +25
10*	0.37; 1.00	4.19	0.776	0.185	1.014	98 ± 13	−23; +31

* Ratio of $\frac{\text{High Dose}}{\text{Low Dose}} = 2.70$.

each set consisting of 4 animals so that a designation $N=5$ corresponds to a total of 20 animals, 10 on the standard and 10 on the unknown.

The known potency ratio, since the standard equals the unknown, is 100 per cent. Actually the potency ratio found varied from 79 ± 16 to 109 ± 15 per cent when a total of 20 to 28 animals were employed. With a total of 20 animals the smallest error range was -20 to $+25$ per cent and the largest error range was -34 to $+52$ per cent. With a total of 28 animals no particular improvement in the error range was found. These error ranges are given at $P=0.95$.

In table 2 where the ratio of high dose to low dose is 2, data similar to that presented in table 1 are illustrated except that 3 runs are included where $N=10$ indicating the use of a total of 40 animals. When $N=10$ the error range was as low as -17 to $+21$ per cent at $P=0.95$.

A third group of comparisons on chorionic gonadotrophin from human pregnancy urine are presented in table 3 where the conditions are similar to the previous data illustrated in the two preceding tables except that the ratio of high dose to low dose is 3. In this series a remarkably close agreement between the theoretical and experimental potency ratios is found. Thus in the seven trials using between 20 to 40 animals per trial the experimental potency ratios vary from 96 ± 10 to 100 ± 16 per cent as compared to the theoretical potency ratio of 100 per cent.

Serum Chorionic Gonadotrophin (Human Pregnancy)

The assay of serum gonadotrophin was studied in a manner similar to that used for the urinary gonadotrophin. It has been previously established that between the limits of 0.0075 and 0.030 cc. of serum equivalent a linear relation could be demonstrated between the logarithm of the dose and the response. Accordingly a series of potency ratios and error ranges were calculated using the Bliss design and various dosage intervals. In each of the cases the standard and unknown were the same. Table 4 summarizes the results when the ratio of the high dose to the low dose was 1.5 .In a total of five comparisons using a total of 20 to 40 immature rats the maximum deviation in the potency ratio was only 4 per cent. The error range of the potency ratio using a total of 40 animals at $P=0.95$ was the remarkably low figure of -7 to $+8$ per cent.

Tables 5 and 6 deal with the assay of chorionic gonadotrophin from serum when the ratios of the high dose to low dose were 2 and 4 respectively. Again the determination of the potency ratio was extremely close to the theoretical value of 100 per cent. At the ratio of 2 the maximum deviation found was 11 per cent while at the ratio of 4, the maximum deviation of 6 per cent was found. In only one of 15

TABLE 4. ASSAY OF CHORIONIC GONADOTROPHIN FROM SERUM BY UTERINE WEIGHT (RAT)

$$\frac{\text{High Dose (Standard)}}{\text{Low Dose (Standard)}} = \frac{\text{High Dose (Unknown)}}{\text{Low Dose (Unknown)}} = 1.5$$

Standard = Unknown

N	Conc. of standard serum	b	s	λ	t	Potency ratio ±S. E. %	Error range $P = 0.95$ %
5	0.020; 0.030	6.02	0.617	0.102	0.503	96 ± 10	−19; +24
5	0.020; 0.030	5.56	0.500	0.090	0.143	100 ± 9.2	−17; +20
7	0.020; 0.030	8.11	0.326	0.040	1.081	99 ± 3.5	− 7; + 7
7	0.020; 0.030	7.92	0.457	0.058	0.338	96 ± 4.8	−10; +11
10	0.020; 0.030	8.10	0.400	0.049	0.675	98 ± 3.5	− 7; + 8

comparisons (tables 5, 6, 7) was a significant difference found in the slopes.

CONCLUSIONS

The assay of human chorionic gonadotrophins either from the urine or from the serum can be adequately assayed by the uterine weight response of the immature rat. The sensitivity of the method is sufficient for most purposes. The blood method can be done on a few cc. of blood while the urinary assay can be adequately performed on as little as 25 cc. of pregnancy urine or about 2 per cent of a day's sample.

The expected accuracy can best be discussed by referring to the summary tables 7 and 8 which are concerned with the urinary and blood chorionic gonadotrophins respectively. In these summary tables we have presented the total number of animals used (equal to

TABLE 5. ASSAY OF CHORIONIC GONADOTROPHIN FROM SERUM BY UTERINE WEIGHT (RAT)

$$\frac{\text{High Dose (Standard)}}{\text{Low Dose (Standard)}} = \frac{\text{High Dose (Unknown)}}{\text{Low Dose (Unknown)}} = 2$$

Standard = Unknown

N	Conc. of standard serum cc.	b	s	λ	t	Potency ratio ±S. E. %	Error range $P = 0.95$ %
5	0.015; 0.030	5.22	0.306	0.058	1.479	110 ± 6.6	−11; +12
5	0.015; 0.030	4.89	0.414	0.085	0.648	97 ± 8.4	−16; +29
7	0.015; 0.030	5.17	0.260	0.050	2.162	95 ± 4.2	− 9, + 9
7	0.015; 0.030	4.90	0.389	0.080	1.065	89 ± 6.2	−13; +15
10	0.015; 0.030	5.06	0.342	0.068	1.495	94 ± 4.6	−11; +11

TABLE 6. ASSAY OF CHORIONIC GONADOTROPHIN FROM SERUM BY UTERINE WEIGHT (RAT)

$$\frac{\text{High Dose (Standard)}}{\text{Low Dose (Standard)}} = \frac{\text{High Dose (Unknown)}}{\text{Low Dose (Unknown)}} = 4$$

Standard = Unknown

N	Conc. of standard serum cc.	b	s	λ	t	Potency ratio ±S. E. %	Error range $P=0.95$ %
5	0.0075; 0.0300	2.83	0.236	0.083	0.066	100 ±8.6	−16; +20
5	0.0075; 0.0300	3.02	0.612	0.202	0.058	102 ±21	−34; +51
7	0.0075; 0.0300	2.81	0.217	0.077	0.129	106 ±7.3	−13; +15
7	0.0075; 0.0300	3.07	0.510	0.166	0.066	97 ±14	−26; +35
10	0.0075; 0.0300	2.91	0.442	0.152	0.244	99 ±10	−19; +24

four times N), the number of trials, the mean potency, the error range at $P=0.95$ in per cent plus or minus the standard error.

From table 7 one can see that true potency ratio of the urinary material can be approached 19 of 20 times with an error of −25 per cent ±1.6 to +36 per cent ±2.6 while increasing the total number of animals to 40 decreases the error range to −19 per cent ±1.1 to +24 per cent ±1.8. The error range of the determination of the potency ratio was even less for the serum gonadotrophin than that for the urinary gonadotrophin. Thus a total of only 20 animals ($N=5$) yielded a mean error range at $P=0.95$ of −19 per cent ±3.5 to +26 per cent ±5.9. Doubling the number of animals to 40 decreased the mean error range to −12 ±3.6 to +14 per cent ±4.0.

The method although being sensitive and reproducible has the disadvantage of a short useful range in the assay curve, that is only two logarithmic intervals. For this reason it is frequently necessary to run a preliminary assay to determine the correct dosage level.

An important precaution in using this method is to be sure that

TABLE 7. SUMMARY OF ASSAY OF CHORIONIC GONADOTROPHIN FROM URINE BY UTERINE WEIGHT (RAT)

Standard = Unknown

Logarithm of Dose-Response Relationship

N	Number of trials	Mean potency ratio %	Mean error range $P=0.95$ +S. E. %
5	15	95	−25 ±1.6; +36 ±2.6
7	6	93	−26 +1.5; +36 ±3.2
10	5	94	−19 ±1.1; +24 ±1.8

the estrogens have been completely removed since they will cause stimulation of the uterus of the immature rat.

Other methods for the assay of human chorionic gonadotrophin have been employed such as the increase in ovarian weight, vaginal introitus and cornification, formation of corpora lutea, increase in testis weight, increase in the seminal vesicles and prostate, and the vaginal cornification of the vitamin B-complex deficient adult rat

TABLE 8. SUMMARY OF ASSAY OF CHORIONIC GONADOTROPHIN FROM SERUM BY UTERINE WEIGHT (RAT)

Standard = Unknown
Logarithm of Dose—Response Relationship

N	Number of trials	Mean potency ratio %	Mean error range $P=0.95$ $\pm$S. E. %
5	6	101	-19 ± 3.5; $+26 \pm 5.9$
7	6	95	-13 ± 1.4; $+15 \pm 4.5$
10	3	97	-12 ± 3.6; $+14 \pm 4.0$

(Heard and Winton, 1939). The report of Emmens (1938) contains description and results of the use of the various methods with the exception of that using the vaginal estrous response of the B-complex deficient adult rat. With this latter method it is possible to determine the potency ratio of an unknown with an error range of -24 per cent to $+31$ per cent at $P=0.99$ using a total of 40 animals. In another study Heard (1941) used the vaginal estrous response of the immature rat and found a somewhat lower accuracy in the determination of the potency ratio. In this latter study the error range of -35 per cent to $+54$ per cent was found at $P=0.99$ when a total of forty rats were used, twenty on the unknown and twenty on the standard.

SUMMARY

A method has been described for the assay of human chorionic gonadotrophins both from urinary sources and from the serum. The method takes advantage of a straight line relationship that can be established between the logarithm of the dose and the response of the immature rat uterus. Using this relationship and the experimental design of Bliss (1944) it was found that the use of a total of 20 animals (10 on the standard and 10 on the unknown) will determine the potency ratio of the urinary material with an accuracy of -25 per cent to $+36$ per cent when $P=0.95$. Increasing the total number of animals to 40 (20 on the unknown and 20 on the standard) decreased the error range to -19 per cent to $+24$ per cent. A somewhat lower error range was found in the determination of the potency ratio of the

serum gonadotrophin. For 20 animals a range of −19 per cent to +25 per cent was found while 40 animals yielded a range of −12 per cent to +14 per cent at $P = 0.95$.

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STUDIES ON THE BIOASSAY OF HORMONES: THE USE OF RADIOSODIUM FOR THE DETECTION OF SMALL QUANTITIES OF DESOXYCORTICOSTERONE[1]

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ALTHOUGH the literature contains numerous references to methods for the evaluation of the influence of adrenal cortical steroids on sodium metabolism no simple method has been suggested which is highly sensitive and reasonably reproducible. We have therefore examined the possibility of using radiosodium for such a method. This communication is concerned with the aspect of sensitivity. The question of reproducibility is still under investigation.

ANIMALS, MATERIALS, AND METHODS

The male albino rats used for these studies were obtained from Carworth Farms in New City, New York. Some animals were bilaterally adrenalectomized in one stage under ether anaesthesia. All animals were exclusively fed a diet of Purina dog chow. If the period between adrenalectomy and the experimental run was more than 24 hours the operated animals received normal saline instead of ordinary drinking water, until the morning of the experiment.

The desoxycorticosterone and desoxycorticosterone acetate were kindly furnished by Ciba Pharmaceutical Products, Inc., Na 24 was purchased from the Monsanto Chemical Co. through the Atomic Energy Commission while the Na 22 was a generous gift from Dr. J. G. Hamilton of the Radiation Laboratory of the University of California. The counting of the radioactive material was done with the Cyclotron Specialties Co. scale of 8 instrument and a G-M tube with a 10 mg./mm.^{2} mica window.

Although some variables of the procedure were modified from experiment to experiment the essential details were similar. In all of the experiments the test material was contained in 0.25 cc. of corn oil injected subcutaneously. After receiving the test material the animals were returned to their cages and given only water. In some experiments a fast of 48 hours was

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[1] Supported in part by grants from Sharp and Dohme, Inc. and the U. S. Public Health Service.

imposed on the animals prior to the administration of the test material.

One hour after the administration of the test material the radiosodium as sodium chloride along with varying amounts of sodium chloride carrier were injected in aqueous solution. This volume was either one or two cc. per animal. After receiving the radiosodium the animals were immediately placed in a glass metabolism cage and the urine was collected quantitatively for 4 or 6 hours depending upon the individual experiment.

The urine was collected in a two-ounce ointment capsule and the metabolism cage was washed with three 10 cc. portions of a 0.1 per cent sodium chloride solution. These conditions of washing were found to collect at least 95 per cent of the excreted material. The urine plus the three 10 cc. washings contained in the ointment capsule were evaporated to dryness by overhead heating using a 300-watt infra red lamp to avoid bumping and splattering. The dried residue was transferred to a suitable holder and the quantity of radiosodium determined.

The radioactive sodium excreted during the experimental period was expressed as the percentage of the total amount administered.

RESULTS

Table 1 illustrates the relative amounts of radiosodium excreted in a test period of 6 hours after the administration of 0.035 mg. of sodium chloride per gram of body weight and no hormone treatment. Within 24 hours after adrenalectomy the rats excreted more than twice as much sodium as during the preoperative period. 3, 5, and 11 days post-operatively the excretion of radiosodium is well above the normal preoperative level.

TABLE 1. EXCRETION OF RADIOSODIUM BY NORMAL AND ADRENALECTOMIZED MALE RATS

(Animals received 0.035 mg. of NaCl per gram of body weight and the urine was collected for 6 hours)

Status of rats	No. of rats	Radiosodium excreted %
Normal	46	0.93
Adrenalectomized 1 day	57	2.14
Adrenalectomized 3 days*	27	2.06
Adrenalectomized 5 days*	8	6.69
Adrenalectomized 11 days*	12	2.48

* Maintained on normal saline up until morning of experiment.

Table 2 summarizes the data on the influence of desoxycorticosterone on the excretion of radiosodium in fasted and fed normal male rats. Some animals were fasted 48 hours while others were normally fed animals. In some instances 0.35 mg. of sodium chloride was administered per gram of body weight while in others the dosage was

TABLE 2. THE INFLUENCE OF DESOXYCORTICOSTERONE ON THE URINARY EXCRETION OF RADIOSODIUM IN NORMAL MALE RATS
(Hormone administered one hour before radiosodium)

Hours of urine collection	Amount of NaCl admin. mg./g. B. W.	Volume of solvent admin. (For NaCl) cc.	Length of fast hr.	Oil treated			Desoxycorticosterone treated			Ratio $\frac{A}{B}$
				No. of rats	Mean body weight g. ± S.E.	(A) amount of Na* excreted % ± S.E.	No. of rats	Amount of DC μg.	Mean body weight g. ± S.E.	(B) amount of Na* excreted % ± S.E.	
4	0.35	1	48	10	51 ± 2.0	4.06 ± 0.18	10	1000	53 ± 2.4	2.92 ± 0.21	1.40
4	0.35	1	48	10	49 ± 1.6	4.31 ± 0.39	10	2000	55 ± 2.2	3.02 ± 0.57	1.43
4	0.035	2	48	10	93 ± 3.1	0.75 ± 0.30	10	2000	104 ± 4.9	0.11 ± 0.04	6.82
4	0.035	2	48	11	90 ± 2.8	0.49 ± 0.21	11	2000	98 ± 3.6	0.06 ± 0.02	8.18
4	0.035	2	48	11	96 ± 5.0	0.29 ± 0.02	11	1000	98 ± 6.1	0.08 ± 0.01	3.63
6	0.035	2	0	11	173 ± 4.2	2.28 ± 0.42	11	500	174 ± 6.2	0.65 ± 0.21	3.51
6	0.035	2	0	11	181 ± 5.7	0.80 ± 0.35	11	250	182 ± 5.5	0.15 ± 0.07	5.33
6	0.035	2	0	12	111 ± 2.2	0.44 ± 0.13	11	250	117 ± 7.6	0.11 ± 0.03	4.00
6	0.035	2	0	12	119	0.48 ± 0.19	10	500	121	0.08 ± 10.02	6.00

* Maintained on normal saline until morning of experiment.

one-tenth of that quantity, 0.035 mg. per gram of body weight. The times of urine collection were 4 and 6 hours respectively and the sodium chloride was administered subcutaneously in one or two cc. of solution at the start of the experimental period. Table 2 also lists the number of animals in each group, the mean body weight, and the mean percentage of excretion of radiosodium and the ratio of excretion of the oil treated animals to the excretion of the hormone treated animals. If no effect has been produced the ratio would be close to one. Values above one indicate retention of the sodium while values below one indicate increased excretion of sodium. In no instance was increased excretion of sodium observed after the administration of desoxycorticosterone.

Although a retention of sodium was found after the administration of 1000 and 2000 micrograms of desoxycorticosterone when 0.35

TABLE 3. THE INFLUENCE OF DESOXYCORTICOSTERONE ON THE URINARY EXCRETION OF RADIOSODIUM IN ADRENALECTOMIZED MALE RATS

(Hormone administered one hour before radiosodium, urine collected for 6 hours, 0.035 mg. of NaCl administered per gram of body weight)

Oil treated			Desoxycorticosterone treated					Ratio A/B
No. of rats	Mean body weight g. ± S.E.	(A) amount of Na* excreted mean % + S.E.	No. of rats	Amount of DC μg.	Mean body weight g. ± S.E.	Interval between operation and experiment days	(B) Amount of Na* excreted mean % ± S.E.	
12	118 ± 8.8	3.56 ± 0.65	10	250	123 ± 3.1	1	0.52 ± 0.38	6.85
12	130 ± 5.4	2.48 ± 0.11	12	125	132 ± 4.0	11	0.48 ± 0.14	5.18
10	146 ± 4.4	2.60 ± 0.50	11	62.5	146 ± 3.5	1	0.10 ± 0.01	26.0
9	132 ± 3.8	1.53 ± 0.22	9	50	133 ± 5.5	1	0.21 ± 0.05	7.27
10	146 ± 4.4	2.60 ± 0.50	11	31.25	144 ± 5.8	1	0.35 ± 0.18	7.43
8	128 ± 3.7	1.48 ± 0.27	9	25	126 ± 3.3	1	0.24 ± 0.09	6.16
9	135 ± 3.4	4.59 ± 0.45	9	25	136 ± 5.5	3	1.26 ± 0.18	3.64
8	139 ± 5.8	6.69 ± 0.55	6	25	141 ± 4.7	5	1.31 ± 0.30	5.10
9	126 ± 1.8	4.30 ± 0.60	8	25 (acetate)	134 ± 3.8	3	0.86 ± 0.19	5.00
9	110 ± 3.1	1.82 ± 0.17	9	25 (acetate)	115 ± 4.2	1	0.17 ± 0.03	10.7
10	155 ± 5.4	1.81 ± 0.33	10	15.63	147 ± 4.4	1	0.07 ± 0.02	25.8
10	155 ± 5.4	1.81 ± 0.33	10	7.82	157 ± 4.4	1	0.14 ± 0.04	12.9
8	175 ± 5.7	1.20 ± 0.20 (Na 22)	7	7.82	183 ± 4.8	1	0.38 ± 0.14 (Na 22)	3.16
8	175 ± 5.7	1.20 ± 0.20 (Na 22)	7	3.91	179 ± 7.3	1	0.44 ± 0.13 (Na 22)	2.73
9	145	2.04 ± 0.33	10	3.91	147	3	1.12 ± 0.35	1.82
9	145	2.04 ± 0.33	11	1.95	138	3	1.35 ± 0.35	1.51
8	175 ± 5.7	1.20 ± 0.20 (Na 22)	7	1.96	177 ± 5.7	1	0.60 ± 0.11 (Na 22)	2.00
8	175 ± 5.7	1.20 ± 0.20 (Na 22)	7	0.98	177 ± 6.3	1	0.55 ± 0.12 (Na 22)	2.18

* Maintained on normal saline until morning of experiment.

milligram of sodium chloride was administered per gram of body weight the response was small. When, however, the amount of sodium chloride was decreased at 0.035 milligram of sodium chloride the amount of retention increased as evidenced by a change in the ratio of 1.40 and 1.43 to 6.82, 8.18, and 3.63 per cent respectively.

The fed animals showed a retention of sodium similar to that observed for the fasted animals, even when the dose of desoxycorticosterone was decreased to 250 micrograms. The length of the urine collection periods was increased from four to six hours in these latter experiments.

An extensive series of experiments were undertaken to ascertain the minimal dose of desoxycorticosterone needed to demonstrate a significant sodium retention in adrenalectomized rats. The results are represented in Table 3. In each case the urine collections were for 6 hours and each animal received 0.035 milligram of sodium chloride per gram of body weight. The interval between operation and the experimental day varied from one to eleven days. As indicated by the ratio significant retention of sodium was found with as little as 0.98 micrograms of desoxycorticosterone. No significant difference in the intensity of retaining effect between various dosages was apparent until the dosage was decreased below 7.82 micrograms. In two instances desoxycorticosterone acetate was employed instead of the free compound without any apparent reduction in activity.

CONCLUSIONS

Although only one adrenal cortical steroid has been studied the method described promises to have a desirable sensitivity. The method promises to detect desoxycorticosterone in the range of one to ten micrograms or the equivalent which is far more sensitive than sodium methods previously used. The sodium method of Hartman *et al.* (1941) which was also studied by Olsen *et al.* (1944), detects about 700 micrograms of desoxycorticosterone acetate in dogs. It was found by the latter workers that this concentration of hormone caused a sodium retention of the order of 35 to 65 per cent although occasionally the percentile retention would fall outside this range.

The 14.8 hour half life of Na 24 presents a large factor of cost if one must depend upon a source of reasonably long distance away from the laboratory. This difficulty may be overcome, however, by the use of Na 22 which has a half life of 3 years. Thus far Na 22 has been extremely difficult to procure.

In all of these studies the reasonable assumptions are made that 1) the total amount of Na present in animals of approximately equal weights, and of the same age and sex and on the same diet is relatively constant and 2) that the administered radiosodium is distributed rapidly throughout the animal.

Since quantities of desoxycorticosterone as low as one microgram

have been found to produce sodium retention, attempts are being made to utilize this method for studies on the adrenotrophic hormone. These studies will be reported at a later time.

The quantitative aspects of this method need further investigation.

SUMMARY

By the use of radiosodium it has been possible to devise a sensitive method for the bioassay of adrenal cortical steroids. Using adrenalectomized rats it has been possible to demonstrate a significant retention in sodium with a little as 0.98 micrograms of desoxycorticosterone. The quantitative aspects of the method as well as the activities of other adrenal cortical steroids remains to be investigated.

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METABOLISM OF THE STEROID HORMONES: STUDIES ON 17-KETOSTEROIDS AND ANDROGENS[1]

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THIS COMMUNICATION is concerned with the influence of gonadectomy, adrenalectomy and the combined operations of gonadectomy and adrenalectomy in male and female monkeys on the urinary excretion of 17-ketosteroids and androgens. The report is also concerned with the possible conversion of desoxycorticosterone and adrenal cortical steroids in whole extracts to 17-ketosteroids in gonadectomized-adrenalectomized monkeys, in an ovariectomized chimpanzee and in four men and a woman.

SUBJECTS, ANIMALS, MATERIALS AND METHODS

The subjects consisted of four patients with Addison's disease, three men and one woman, and one hypogonadal male. Clinical studies on the four Addison's disease patients have been published (Shipley, 1944). Five male and four female rhesus monkeys were used. Four of the animals had been in the laboratory for about four years and were in good health. The remaining five were purchased from animal dealers and were in good health on arrival at the laboratory. None of the nine monkeys showed signs of respiratory disease at autopsy. Prior to adrenalectomy, the monkeys were maintained on Purina Dog Chow supplemented by fresh cabbage and oranges. After adrenalectomy, they usually lost their appetite for the dog chow and then subsisted on oranges, apples, carrots, cabbage, potatoes, eggs and normal saline. The animals were kept in metabolism cages and the urine collected daily under toluene.

Bilateral orchiectomy or ovaridectomy was performed in one stage while adrenalectomy was performed in two stages. Ether anaesthesia was used for all operations. The adrenalectomized animals all died spontaneously after withdrawal of hormone treatment. Completeness of adrenalectomy was checked at autopsy. No complicating disease was present in any of the animals.

The ovariectomized chimpanzee used in this study was a member of the Orange Park Colony in Orange Park, Florida. All urines were collected in Orange Park and promptly shipped to Cleveland for processing.

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The desoxycorticosterone[2] was administered in the form of its acetate in capsules when administered orally, in peanut oil when administered intramuscularly, and in pellets when implanted subcutaneously. Three types of adrenal cortical extracts were employed, all made by Wilson Laboratories. One was the usual commercial extract which was administered either intramuscularly, intravenously or intraperitoneally. The other two extracts consisted of extracts prepared for oral administration with 50% glycerol and concentrated 1.2 and 1.8 times respectively as compared to the ordinary commercial extract.

The urines were collected on a twenty-four-hour basis and simultaneously hydrolyzed with hydrochloric acid and extracted with carbon tetrachloride by the method previously described (Fish, Horwitt and Dorfman, 1943). The Holtorff and Koch (1940) method for the determination of 17-ketosteroids was employed: The use of the term 17-ketosteroid used throughout this paper does not imply that the experimental values represent only 17-ketosteroids. Actually the presence of either 3- or 20-ketosteroids would also influence the magnitude of the 17-ketosteroids values. However, on a molar basis the contribution of either 3- or 20-ketosteroids would be small as compared to 17-ketosteroids. Determinations of 17-ketosteroids were made on the ketonic neutral fraction, prepared from the total neutral fraction with the Girard-Sandulesco reagent T. In the case of the chimpanzee urine, the extracts were further fractioned into the ketonic hydroxy and ketonic non-hydroxy fractions by use of succinic acid anhydride.

Assays for androgenic material were done by the method of Frank *et al.* (1942). This method utilized the sensitivity of the baby chick comb to the inunction of androgens. All results were expressed in international units (I.U.) per day.

EXPERIMENTAL

THE EXCRETION OF 17-KETOSTEROIDS AND ANDROGENS IN MONKEYS. Table 1 is a summary of concentrations of 17-ketosteroids and of androgens in the urine of 5 male and 4 female intact monkeys. The mean value for 17-ketosteroids in the males was found to be 2.1 ± 0.3 mg. per day as compared to 1.2 ± 0.1 mg. for the females. The values for androgens were 2.0 I.U. per day for the males as compared to 1.6 I.U. per day for the female monkeys. These values for androgens are in good agreement with those previously reported for adult male and female monkeys (Dorfman and Van Wagenen, 1941), which were respectively 2.6 and 2.2 I.U. per day.

Influence of Ovariectomy.

The influence of ovariectomy on 17-ketosteroid excretion was studied in 2 monkeys. Monkey 2 was followed from the 1st to 49th day postoperatively while monkey 6 was studied only for the first 5 days following ovariectomy (table 2). The two animals present quite different pictures of 17-ketosteroid excretion after operation even though the initial preoperative levels were quite similar. Monkey

[2] The desoxycorticosterone acetete was kindly furnished by Ciba Pharmaceutical Company, Inc.

2 excreted 1.1 ± 0.08 mg. per day, while monkey 6 excreted 1.0 ± 0.06 mg. per day. During the first 5 postoperative days, the 17-ketosteroid excretion of monkey 2 increased from 1.1 ± 0.08 to a value of 3.3 mg. on the 5th day. The mean value of 2.6 mg. for the first 6 days represented a rise of greater than 200%. Monkey 6, however, showed no such increases. Actually the mean value of 0.8 ± 0.05 found during the 5 postoperative days represented a slight decrease in 17-ketosteroid excretion.

TABLE 1. THE EXCRETION OF 17-KETOSTEROIDS AND ANDROGENS BY INTACT MALE AND FEMALE MONKEYS

Animal number & sex	Weight kg.	Number of samples	Number of days	Ketonic-neutral fraction	
				Ketosteroids Mg./day ±S. E.	Androgens I.U./day
1 ♂	8.4	13	19	2.1 ±0.12	
3 ♂	7.6	6 1	16 4	1.4 ±0.14	2.2
4 ♂	6.9	6	20	2.4 ±0.27	
5 ♂	11.0	6 1	12 4	2.8 ±0.25	2.6
8 ♂	5.9	5 1	11 4	1.6 ±0.33	1.2
2 ♀	5.2	13 1	19 6	1.1 ±0.08	2.8
6 ♀	4.5	7 1	15 5	1.0 ±0.06	1.0
7 ♀	6.6	4	9	1.2 ±0.08	
9 ♀	4.2	7 1	17 6	1.4 ±0.26	1.0
		Means:	♂	2.1 ±0.3	2.0
			♀	1.2 ±0.1	1.6

Unfortunately, no figures are available for the changes between the 6th and 20th postoperative days for monkey 2, but by the 20th day, the urinary 17-ketosteroid concentration is decreased. Between the 22nd and 49th days, the 17-ketosteroid excretion was back to the preoperative level.

Influence of Castration.

Three male monkeys (numbers 3, 4 and 8) were castrated and followed for a period of approximately 4 weeks postoperatively (table 2). Initial increases were evident within the first three days in monkeys 3 and 4. However, monkey 8 showed no such increases. By the third postoperative week, distinct drops were in evidence in all animals. Decreases in 17-ketosteroid excretion at this time were

43%, 63% and 50% respectively for monkeys 3, 4 and 8. Thus an average decrease of 52% was observed in the monkeys studied.

TABLE 2. THE INFLUENCE OF GONADECTOMY, ADRENALECTOMY AND GONADECTOMY-ADRENALECTOMY ON THE EXCRETION OF 17-KETOSTEROIDS IN MALE AND FEMALE MONKEYS

(All values are in mg./day)

Monkey number and sex	Intact +S. E.	Status of monkey			
		Gonadectomized		Adrenalectomized	Gonadectomized and adrenalectomized
		First week	Second week or longer		
2 ♀	1.1 ±0.08 13	2.6 (1.9–3.3)[1] 5[2]	1.0 (0.9–1.4) 5		0.5 (0.2–1.0) 4
6 ♀	1.0 ±0.06 7	0.8 (0.6–1.1) 5			0.3 (0.0–0.5) 8
9 ♀	1.4 ±0.26 8			0.3 (0.1–0.4) 4	
3 ♂	1.4 ±0.14 6	1.9 (1.1–2.3) 3	0.9 (0.8–1.0) 3		0.6 (0.5–0.7) 3
8 ♂	1.6 ±0.27 5	1.2 (1.0–1.3) 2	0.8 (0.8–1.0) 3		0.4 (0.2–0.9) 6
4 ♂	2.4 ±0.27 6	3.6 (2.5–4.7) 2	1.1 (0.8–1.7) 3		
5 ♂	2.8 ±0.25 6			0.6 (0.4–1.0) 5	

[1] Range of values.
[2] Number of samples.

Influence of Adrenalectomy.

Male monkey 5 was adrenalectomized and followed from the 2nd to 5th day postoperative, and one sample run at 21 days. The mean value for the 5 days studied was 0.6 mg. as compared to the control value of 2.8 ± 0.25 mg., or a decrease of 79% (table 2). Similar results were obtained after bilateral adrenalectomy for female monkey 9. Here a decrease of 71% was noted in a change from 1.4 ± 0.26 to 0.3 mg. per day (table 2).

Influence of Gonadectomy and Adrenalectomy.

Four samples representing 9 days of urine were collected after the removal of one adrenal from monkey 2 which had been previously ovariectomized (table 2). During the 13 day period after the removal of one adrenal, a small decrease in 17-ketosteroids was observed. Thus

while intact, this animal excreted 1.1 ± 0.08 mg., and during the period from the 22nd to 49th day after ovariectomy, the value was 1.0 mg. However, after removal of the one adrenal, the excretion decreased to 0.8 mg. per 24 hours.

Although the removal of the second adrenal from monkey 2 (table 2) produced a decrease in 17-ketosteroid excretion, a significant amount of steroid was still found. Four samples were collected representing 6 days between the 27th to 46th days after the last operation.

During this period, the animal excreted 0.5 ± 0.2 mg. representing 55% of the original excretion. Monkey 6, after ovarietcomy and adrenalectomy, presented a similar picture (table 2). Eight samples representing 11 days were collected from the 1st to 41st day. A mean of 0.3 ± 0.1 mg. was found representing 30% of the concentration of urinary 17-ketosteroids originally present.

Two male monkeys, 3 and 8, were both castrated and adrenalectomized (table 2). Here again definite 17-ketosteroid excretion was observed even after both glands were removed. The urinary concentration of monkey 8 showed a decrease from 1.6 ± 0.33 mg. to 0.4 mg. after the two operations. Thus a decrease of 75% occurred. Similar results were found in the case of monkey 3 where a decrease of 57% was observed from 1.4 ± 0.14 mg. to 0.6 mg.

In view of the fact that 17-ketosteroid excretion seemed to be still in evidence after removal of both gonads and adrenals in male and female monkeys, it was of interest to see if a more specific test, a test for androgens, would be positive. Assays for androgenic activity were done on three male and three female monkeys.

The androgenic material in males averaged 2.0 I.U. per day as compared to 1.6 I.U. per day for the females (table 3).

TABLE 3. THE INFLUENCE OF GONADECTOMY, ADRENALECTOMY, AND GONADECTOMY-ADRENALECTOMY ON THE EXCRETION OF ANDROGENS IN MALE AND FEMALE MONKEYS

(All values in I.U. per day)

Number and sex	Weight K.	Intact	Gonadectomized	Adrenalectomized	Gonadectomized and Adrenalectomized
2 ♀	5.2	2.8	1.5		0.4
6 ♀	4.5	1.0	1.0		0.6
9 ♀	4.2	1.0		0.4	
3 ♂	7.6	2.2	1.8		0.7
8 ♂	5.9	1.2	0.7		0.5
5 ♂	11.0	2.6		1.8	

Removal of the ovaries of female monkey 2 resulted in a decrease of 46% in excretion of androgenic material, while ovariectomy in a

second monkey had no influence on the androgenic titer. Castration of monkeys 3 and 8 caused decreases of 18% and 42% respectively in androgen excretion.

The removal of the adrenals in either female monkey 9 or male monkey 5 caused distinct drops in androgens. These changes were 31% for the female and 60% for the male.

Androgens were excreted after removal of both gonads and adrenals in male and female monkeys. Thus 0.4 I.U. per day and 0.6 I.U. per day was found after both operations for female monkeys 2 and 6 respectively. This amounted to 14% and 60% of the original concentration of androgens present in the urine of the intact animals. A similar situation was found in the case of the males. After the double operation, male monkey 3 still excreted 30% of the original concentration of androgens and male monkey 8 excreted 42%.

TABLE 4. THE EXCRETION OF 17-KETOSTEROIDS AND ANDROGENS AFTER GONADECTOMY AND ADRENALECTOMY IN MALE AND FEMALE MONKEYS

Number of animals	Sex	Androgens % of preoperative value (mean)	17-Ketosteroids % of preoperative value (mean)
2	♀	37	38
2	♂	37	29

Table 4 is a comparison of the decreases observed in both androgens and 17-ketosteroids after gonadectomy and adrenalectomy. The magnitude of decreases for both androgens and 17-ketosteroids was similar in both male and female monkeys.

THE EXCRETION OF 17-KETOSTEROIDS AFTER THE ADMINISTRATION OF DESOXYCORTICOSTERONE AND ADRENAL CORTICAL EXTRACTS.

Ovariectomized Chimpanzee.

A summary of the influence of orally administered desoxycorticosterone acetate on the urinary excretion of 17-ketosteroids in the bilaterally ovariectomized chimpanzee is represented in table 5. After a pretreatment period when 13 days of urine was collected, 100 mg. of desoxycorticosterone acetate was administered daily for 12 days. The urine was collected during the administration period and for the four following days. During the period of hormone administration, increases in the 17-ketosteroids of both the ketonic-hydroxy and ketonic-nonhydroxy fractions were observed. The former fraction increased from 0.48 to 1.00 mg. per day while the latter fraction showed an increase from 1.15 to 4.00 mg. per day. In a second experiment, the

TABLE 5. THE EXCRETION OF 17-KETOSTEROIDS AFTER THE ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE IN THE OVARIECTOMIZED CHIMPANZEE

Desoxycorticosterone acetate		Urine collection days	Total vol. of urine liters	17-Ketosteroids	
Total dose orally gm.	Days of administration			Ketonic hydroxy fraction mg./day	Ketonic non-hydroxy fraction mg./day
0	0	13	8.9	0.48	1.15
1.2	12	16	10.7	1.00	4.00
3.0	15	18	15.1	1.19	4.55

daily dose was increased to 200 mg. per day and administered for 15 consecutive days. Urine was collected for the 15 days during hormone administration and for the following three days. This second period of desoxycorticosterone acetate administration again demonstrated increased urinary 17-ketosteroid titers for both the ketonic-hydroxy and ketonic-nonhydroxy fractions.

Gonadectomized and Gonadectomized-Adrenalectomized Monkeys.

Table 6 lists 4 metabolism experiments on a gonadectomized-adrenalectomized monkey which still excreted a small quantity of 17-ketosteroids, 0.5 ± 0.2 mg. per 24 hours. Experiments I, II, III, in which various quantities of adrenal cortical extract were administered, showed definite conversions of adrenal cortical steroids to 17-ketosteroids. In experiment I, the administration of 25 cc. of adrenal cortical extract caused a slight increase in 17-ketosteroid excretion during the 1st day and a greater increase in the 2nd day, that is a value of 2.0 mg. per day was found. By the third day, or two days after stopping treatment, the 17-ketosteroid excretion was down to the pretreatment level of 0.5 mg. Similar results were obtained in experiments II and III. If we assume that the control level of 17-ketosteroid remains constant and further that the steroid content of adrenal cortical extract is equal to 1 mg. per cc., the over-all percentage excretion of administered material was 8% for experiment I, 11.4% for experiment II and 12.2% for experiment III.

When 60 mg. of desoxycorticosterone acetate was administered over a 4 day period, the average excretion during the four days was increased slightly from 0.5 mg. to 1.0 mg. During the three consecutive days after treatment, the values were 2.2, 2.0 and 1.7 mg. per day respectively. Collections were stopped after the third post-treatment day. The total 17-ketosteroid excretion during the treatment period and for the following three days amounted to 9.9 mg. If we assume the endogenous excretion remained constant during this experiment

TABLE 6. THE EXCRETION OF 17-KETOSTEROIDS AFTER THE ADMINISTRATION OF ADRENAL CORTICAL EXTRACT AND DESOXYCORTICOSTERONE ACETATE TO MONKEY 2

(Ovariectomized and Bilaterally Adrenalectomized)

Exp. No.	Treatment			No. days treatment	No. days since treatment	No. days of sample	17-Ketosteroids in ketonic neutral fraction mg./day
	Adrenal cortical extract		Desoxycorticosterone acetate I.M. mg.				
	I.V. cc.	I.M. cc.					
	0	0	0	—	—	6	0.5 ±0.2
I	20	5	0	1	—	1	1.0
	0	0	0	0	1	1	2.0
	0	0	0	0	2	1	0.5
II	20	0	0	1	—	1	1.0
	0	0	0	0	1	1	2.4
III	25	0	0	1	—	1	2.5
	25	0	0	1	—	1	
	0	0	0	0	1	1	2.6
IV	0	0	10	1	—	1	1.0
	0	0	10	1	—	1	
	0	0	20	1	—	1	
	0	0	20	1	—	1	
	0	0	0	0	1	1	2.2
	0	0	0	0	2	1	2.0
	0	0	0	0	3	1	1.7

period, the net gain in 17-ketosteroid amounted to 6.4 mg. This would be equal to a recovery of 13.8% of the administered desoxycorticosterone acetate. Table 7 lists 2 recovery experiments in which adrenal cortical extract was administered to a second ovariectomized-adrenalectomized monkey. When 10 cc. doses of adrenal cortical extract were administered on two successive days, the increased excretion of 17-ketosteroids was small. The intraperitoneal administration of 50 cc. of adrenal cortical extract caused a significant rise in 17-ketosteroids during the day of administration and for the subsequent day. On the basis of our previous assumptions, a recovery of 4.2% was realized.

Table 8 lists 2 experiments in which desoxycorticosterone and adrenal cortical extracts were administered to an adrenalectomized male monkey. Small increases in 17-ketostroid excretion were obeserved.

TABLE 7. THE EXCRETION OF 17-KETOSTEROIDS AFTER THE ADMINISTRATION OF ADRENAL CORTICAL EXTRACT TO MONKEY 6

(Ovariectomized and Adrenalectomized)

Exp. No.	Treatment			No. days since treatment	No. days of sample	17-Ketosteroids in ketonic-neutral fraction mg./day
	Adrenal cortical		Desoxycorcosterone I.M. mg.			
	I.V. cc.	I.P. cc.				
	0	0	0	—	11	0.3 ± 0.1
I	10	0	0	—	1	0.4
	10	0	0	—	1	0.8
II	0	50	0	—	1	1.4
				1	1	1.3
				2–3	1	0.5
				4–5	1	0.2
III	0	25	0	—	1	1.0

TABLE 8. THE EXCRETION OF 17-KETOSTEROIDS AFTER THE ADMINISTRATION OF ADRENAL CORTICAL EXTRACT AND DESOXYCORTICOSTERONE ACETATE TO MONKEY 5

(Adrenalectomized)

Exp. No.	Treatment		No. days treatment	No. days since treatment	No. days of sample	17-Ketosteroids in ketonic-neutral fraction mg./day
	Adrenal cortical extract I.V. cc.	Desoxycorticosterone acetate I.M. mg.				
	0	0	0	0	5	0.6 ± 0.4
I	0	10	1	—	1	
	0	10	1	—	1	1.0
	0	5	1	—	1	
II	25	0	1	—	1	1.0
	25	0	1	—	1	
				1	1	0.6

Men and Women.

The experimental details and results on Patient 1 are represented in table 9. Patient 1, a 28 year old male patient with Addison's disease, received adrenal cortical extract intramuscularly and desoxycorticosterone acetate both by intramuscular administration and implantation. Fourteen samples representing a total of 19 days of urine were collected. These samples represent urines collected before and during various forms of treatment.

TABLE 9. THE EXCRETION OF 17-KETOSTEROIDS AFTER THE ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE AND ADRENAL CORTICAL EXTRACT

Patient 1

(J.B.—Male, Addison's Disease, Age 28 years)

Desoxycorticosterone Acetate		Adrenal cortical extract intramuscularly—cc.	Number of days	17-Ketosteroids ih ketonic fraction mg./day
Intramuscularly mg.	Pellets mg.			
0	0	0	4	0.7
5	0	10	1	2.2
25	0	0	5	2.8
0	150	0	3*	4.0
0	0	0	4**	2.3
0	450	0	10***	2.4

* First three days following implantation of 150 mg. of desoxycorticosterone acetate.

** Urine collected on 130–132nd and 134–136th days following implantation of 150 mg. of desoxycorticosterone acetate.

*** Urine collected from 133 to 143rd day following implantation of 450 mg. of desoxycorticosterone acetate.

Three samples of urine were collected during a four day period when the patient received no treatment. Though the 17-ketosteroids determined on the total neutral fraction varied from 1.3 to 4.0 mg. per day, the values found for the ketonic neutral fraction varied only from 0.5 to 0.8 mg. per day. Six samples of urine were collected representing seven days during which time 5 mg. of desoxycorticosterone acetate per day was administered intramuscularly. On the first day of treatment 10 cc. of adrenal cortical extract was also administered intramuscularly. During this period, an increased excretion of 17-ketosteroids was found. During the first day of treatment the value increased to 2.2 mg. while during the following 5 days of treatment the excretion averaged 2.8 mg. After the implantation of 150 mg. of desoxycorticosterone acetate pellets, 5 samples of urines were collected, distributed from the 1st to the 135th day after implantation. Here again definite increases in 17-kestosteroids were found. Similar results were obtained during a second period when 450 mg. of desoxycorticosterone pellets were implanted.

The experimental details on patient 2 are noted in table 10. Five 24 hour urine samples were collected before any adrenal cortical substances were administered. The values of 17-ketosteroids in the total neutral fraction varied from 2.4 to 3.6 mg. per 24 hours with an average of 3.1 mg. per 24 hours. The average 17-ketosteroid value for the ketonic neutral fraction, however, was only 0.6 mg. per day. Dur-

ing an experimental period of 11 days, the patient received 1450 mg. of desoxycorticosterone acetate orally in amounts of 100 and 150 mg. per day. On 6 of the 11 days, the patient received intramuscularly between 5 to 15 cc. of adrenal cortical extract. This treatment caused an increase in 17-ketosteroids in the ketonic fraction from 0.6 mg. per day to 1.9 mg. per day. A much more striking increase in 17-ketosteroids was found after the oral administration of 270 cc. of an ad-

TABLE 10. THE EXCRETION OF 17-KETOSTEROIDS AFTER THE ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE AND ADRENAL CORTICAL EXTRACT

Patient 2

(M.W.—Female, Addison's Disease, Age 30 years)

Desoxycorticosterone acetate		Adrenal cortical extract			No. of days	17-Ketosteroids in ketonic fraction mg./day
Orally mg.	Pellets mg.	Orally cc.	Intramuscularly cc.	Intravenously cc.		
0	0	0	0	0	5	0.6
1450	0	0	60	0	11	1.9
0	0	270	0	0	5	5.1
0	0	0	0	0	1	1.9*
0	300	0	0	20	1	2.8†
0	300	0	0	0	4	1.4**

* Three days after discounting oral extract.

** These values represent average values for the four days following implantation of 300 mg. of desoxycorticosterone acetate.

† Day of desoxycorticosterone acetate implantation.

renal cortical extract during a 4 day period. During this experimental period the average 17-ketosteroid excretion as measured in the ketonic neutral fraction increased to 5.1 mg. per day. Three days after discontinuing the oral treatment, the excretion of 17-ketosteroids decreased to 1.9 mg. per day. After implantation of 300 mg. of desoxycorticosterone acetate, values of 2.8 and 1.4 mg. per day were found for the day of implantation and four following days respectively.

Patient 3 (table 11) was studied during the following periods: pretreatment period; oral treatment with desoxycorticosterone acetate; sulfadiazine, desoxycorticosterone acetate and adrenal cortical extract treatment for pneumonia and adrenal insufficiency; intravenous adrenal cortical extract and intramuscular desoxycorticosterone acetate; and finally oral administration of adrenal cortical extract. Three pretreatment 24 hour urine samples were found to contain average 17-ketosteroid values of 3.8 and 2.4 mg. per day respectively for the total neutral and ketonic neutral fractions. These

TABLE 11. THE EXCRETION 17-KETOSTEROIDS AFTER THE ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE AND ADRENAL CORTICAL EXTRACT

Patient 3

(A.G.—Male, Addison's Disease, Age 34 years)

Sulfadiazine mg.	Desoxycorticosterone acetate			Adrenal cortical extract		Number of days	17-Ketosteroids in ketonic fraction mg./day
	Orally mg.	Intramuscularly, mg.	Pellets mg.	Orally cc.	Intravenously cc.		
0	0	0	0	0	0	3	2.4
0	700	0	0	0	0	6	3.0
38.5	0	105	0	0	160	11	2.9
0	0	10	0	648	0	8	4.3
0	0	0	0	126	0	1	5.8*
0	0	0	0	0	0	1	4.0**
0	0	0	300	0	0	5	2.4†

* Fresh urine extracted first with ethylene dichloride, then extracted in the usual manner with carbon tetrachloride.

** Forty-eight hours after last oral administration.

† Urine collected on 30, 31, 32, 38 and 39th day following implantation of 300 mg. of desoxycorticosterone acetate.

values were only slightly increased during the 6 day period when 700 mg. of desocycorticosterone acetate was administered orally. The 17-ketosteroid concentration in the ketonic neutral fraction was 3.0 mg. per 24 hours. During an 8 day period when 774 cc. of adrenal cortical extract was administered orally, the 17-ketosteroids were increased to 4.3 mg. Two days after this treatment period, the concentration was still 4.0 mg. per day.

Table 12 lists the studies on Patient 4, a male Addison's disease patient, age 33 years. The pretreatment period of 9 days indicated an excretion of 6.7 mg. of 17-ketosteroids per day in the ketonic neu-

TABLE 12. THE EXCRETION OF 17-KETOSTEROIDS AFTER THE ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE AND ADRENAL CORTICAL EXTRACT

Patient 4

(R.P.—Male, Addison's Disease, Age 33 years)

Desoxycorticosterone acetate	Adrenal cortical extract	Number of days	17-Ketosteroids in ketonic fraction mg./day
Orally mg.	Orally cc.		
0	0	9	6.7
750	0	6	6.3
0	780	5	12.5

tral fraction. The oral administration of 750 mg. of desoxycorticosterone acetate over a 6 day period produced no increase in 17-ketosteroid excretion. The oral administration of 780 cc. of adrenal cortical extract over a 5 day period increased the excretion of 17-ketosteroids from 6.7 mg. to 12.5 mg. per day.

Patient 5, a hypogonadal male, received a total of 850 mg. of desoxycorticosterone acetate during a 7 day period. No increase in 17-ketosteroids was observed. (Control value 11.6 mg., during desoxycorticosterone acetate 10.0 mg. per day.)

DISCUSSION

The experimental results indicate the possibility of an undescribed source of 17-ketosteroids in addition to those already known, the adrenals and gonads. The fact that the test for 17-ketosteroids is still positive after removal of both gonads and adrenals is perhaps not too striking. However, the fact that these doubly operated animals still excrete androgenic material is indeed striking. What about accessory adrenal tissue? A final answer is, of course, impossible to give, but it must be said that each of the four monkeys which were gonadectomized and adrenalectomized did die of adrenal insufficiency. In many instances these adrenalectomized animals went into adrenal crisis and were revived with adrenal cortical extract and saline. Adrenal tissue could not be found at autopsy, and, finally, assays of the urines of these monkeys for cortin-like material were invariably negative (Dorfman *et al.*, 1944).

There is a possibility that this small excretion of 17-ketosteroids and androgens is of exogenous origin. Up to the present, however, no androgenic material has been demonstrated in plant material, the type of material on which for the most part the monkeys subsisted.

The question of the excretion of 17-ketosteroids after gonadectomy is indeed complicated. The results bearing on the influence of castration in male monkeys point to a decreased excretion, but only after several days have elapsed since operation. Thus a mean decrease of some 52% in three cases was observed. During this time, the androgen excretion also decreased in two cases studied, one 18% and the other 42%. During the first 3 to 5 days following castration, 2 of the 3 male monkeys showed definite increases in 17-ketosteroid excretion. These increases presumably were due to compensatory hypersecretion by the adrenal cortex.

In the two female monkeys studied after ovariectomy, one monkey showed a distinct rise in 17-ketosteroid excretion during the first week postoperatively while the other did not. However, the 17-ketosteroid excretion, in the one animal in which urines were studied through the 49th day after operation, did return to the preoperative level.

Studies on the effect of adrenalectomy on the excretion of 17-ketosteroids were not particularly helpful in evaluating the relative

importance of the 17-ketosteroid precursors in the gonads. Actually, the decrease observed was as great after adrenalectomy as after gonadectomy and adrenalectomy. Even though the adrenalectomized animals were in apparently good condition, being maintained on salt during the period of urine collections, the gonads were probably not functioning optimally.

Table 13 summarizes the calculated conversion of orally administered desoxycorticosterone to 17-ketosteroids. For the purpose of the calculation, the assumption is made that the pretreatment control level of urinary 17-ketosteroids remained constant during the treatment period. Further, if desoxycorticosterone were metabolized to a 17-ketosteroid, such as androsterone, a correction should be applied in calculating the percentage recovery to correct for the difference in molecular weight. Thus, in the conversion of desoxycorticosterone to androsterone the theoretical conversion would be represented by the ratio 274 to 314.

TABLE 13. THE URINARY EXCRETION OF 17-KETOSTEROIDS AFTER THE ORAL ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE

Subject	No. days treatment	Desoxycorticosterone acetate calculated as free compound mg.	The recovery of 17-ketosteroids			
			Ketonic fraction (during treatment) mg.	Ketonic fraction (control value) mg.	Net gain in ketone fraction mg.	Conversion %
Patient 2 ♀ Addison's Disease	11	1280	20.9	6.6	14.3	1.3
Patient 3 ♂ Addison's Disease	6	621	17.8	14.4	3.4	0.7
Patient 4 ♂ Addison's Disease	6	665	37.7	43.2	−5.4	−0.8
Patient 5 ♂ Hypogonad	7	754	70.0	81.2	−11.2	−1.5
Ovariectomized Adult	16	1060	80.0	26.1	53.9	5.8
Chimpanzee	18	2660	103.3	29.3	74.0	3.2
Ovariectomized Adrenalectomized Monkey	4	53	9.9	3.5	6.4	13.8

In the case of the male hypogonad, no increase in 17-ketosteroids was observed. In the case of the female Addison's disease patient (Patient 2), a 1.3% conversion was found as compared to 0.7% in the case of one male Addison's disease patient (Patient 3), and −0.8% in a second male Addison's disease patient. The largest conversions

were observed in the 2 experiments involving oral administration of desoxycorticosterone to an ovariectomized chimpanzee, and one experiment involving desoxycorticosterone acetate administration to an ovariectomized-adrenalectomized monkey. Conversions of 5.8% and 3.2% respectively for two experiments were found for the ovariectomized chimpanzee, while in the case of the doubly operated monkey 13.8% conversion was observed.

The small percentage conversion of desoxycorticosterone acetate to 17-ketosteroids in some of the Addison's disease patients may be only an apparent conversion. Thus the gonads during the pretreatment period may be producing a reduced quantity of 17-ketosteroid precursors due to the poor general condition of the patient. After treatment, however, the gonads may show increases in the production

TABLE 14. THE EXCRETION OF 17-KETOSTEROIDS AFTER THE IMPLANTATION OF PELLETS OF DESOXYCORTICOSTERONE ACETATE

Addison's Disease patient	Number of days	Desoxycorticosterone		Recovery of 17-ketosteroids			
		Acetate implanted calculated as free compound mg.	Amount absorbed (free compound)	Ketonic fraction (during implantation) mg.	Ketonic fraction (control value) mg.	Net gain in ketonic fraction mg.	Conversion
1 ♂	136	264	88	312.8	95.2	217.6	280
1 ♂	143	396	132	343.2	100.1	243.1	208

of 17-ketosteroid precursors which would be reflected in an increased titer of 17-ketosteroids.

In the case of the studies involving the ovariectomized chimpanzee, the conversion of desoxycorticosterone to 17-ketosteroids seems to be real. Since the dosage of desoxycorticosterone was quite high, that is, 100 and 200 mg. per day respectively, it is quite likely that the production of 17-ketosteroid precursors by the animal's own adrenal was decreased and that the total conversion of desoxycorticosterone to 17-ketosteroid is greater than appears, since the endogenous level of 17-ketosteroids is probably lower during treatment than during the pretreatment period. The intramuscular administration of desoxycorticosterone to the ovariectomized-adrenalectomized monkey caused a real increment in 17-ketosteroid.

We have already mentioned the possibility that at least a portion of the increased 17-ketosteroid excretion after desoxycorticosterone administration to Addison's disease patients may be due to the better condition of the patient and subsequent increased production of gonadal 17-ketosteroid precursors. Experiments summarized in table 14 seem to illustrate this point. After the implantation of desoxycorticosterone acetate pellets, a number of urine samples were collected over a period of 134 days for Patient 1 in one experiment,

and for 143 days for Patient 1 in a second experiment. Under the conditions of these experiments, 280% and 208% conversions of desoxycorticosterone to 17-ketosteroids was observed. The increased 17-ketosteroid excretion under these conditions is far in excess of the theoretical amount expected. Obviously, the increased amount must have come from a source other than the administered desoxycorticosterone and most likely the gonads.

Since none of the adrenal cortical steroids other than desoxycorticosterone were available in sufficient amounts to study their metabolism, studies on the metabolism of adrenal cortical extracts were done. The administration of adrenal cortical extracts containing mixtures of steroids caused distinct rises in 17-ketosteroids.

Larger increases in urinary 17-ketosteroids were observed after the administration of adrenal cortical extracts to Addison's disease patients than were observed for desoxycorticosterone (table 15). Certain difficulties arise in attempting to evaluate the percentage

TABLE 15. THE URINARY EXCRETION OF 17-KETOSTEROIDS AFTER THE ORAL ADMINISTRATION OF ADRENAL CORTICAL EXTRACTS

Addison's Disease patient	No. days of treatment	Adrenal cortical extract	Maximum weight of steroids	Recovery of 17-ketosteroids			
				Ketonic fraction (during treatment)	Ketonic fraction (Control value)	Net gain in ketonic fraction	Conversion minimum
		cc.	mg.	mg.	mg.	mg.	%
2 ♀	5	270	270	30.7	4.8	25.9	9
3 ♂	9	774	774	40.2	21.6	18.6	2.4
4 ♂	5	780	780	62.5	36.0	26.5	3.4

conversion of steroids in whole extracts to 17-ketosteroids. No adequate analysis is available of the concentration of steroids in the extracts employed. However, it was possible to calculate minimum recoveries by using the weight of the total solids of the extracts. The maximum weight of steroids for the extracts employed was 1 mg. per cc. On that basis, minimum conversions of 2.4, 3.4, and 9% were observed in the 3 Addison's disease patients studied. These calculations do not correct for the fact that most of the adrenal cortical steroids have a greater molecular weight than the 17-ketosteroids. If this were done the minimum conversion values would be larger.

The administration of adrenal cortical extracts to the Addison's disease patients produced distinct increases in 17-ketosteroid excretion; we have again the complicating factor of the gonads. It may be argued that the increased excretion was actually due to gonadal precursors being produced in increased amounts after treatment with adrenal cortical extract.

The recovery experiments on the adrenalectomized-gonadectomized monkeys more clearly illustrate the conversion of adrenal cortical steroids contained in the extracts, to 17-ketosteroids. Thus in all six experiments involving the administration of adrenal cortical extracts to these doubly operated animals, increases in 17-ketosteroid excretion were observed; in some cases these increases amounted to as much as 500% of pretreatment values. Table 16 lists the minimum percentage conversions of adrenal cortical steroids to 17-ketosteroids, calculated in a manner similar to those listed in Table 13. The minimum conversions ranged from 2.8% to 12.2%.

TABLE 16. THE URINARY EXCRETION OF 17-KETOSTEROIDS AFTER THE ADMINISTRATION OF ADRENAL CORTICAL EXTRACTS TO OVARIECTOMIZED-ADRENALECTOMIZED MONKEYS

Monkey	No. of days of treatment	No. of days of urine	Adrenal cortical extract steroids cc.	Maximum weight of ment) mg.	Recovery of 17-Ketosteroids			
					Ketonic fracton (during treat- mg.	Ketonic fraction (control value) mg.	Net gain in ketonic fraction mg.	Conversion minimum %
2	1	2	25 (I.V.)	25	3.0	1.0	2.0	8
	1	2	21 (I.V.)	21	3.4	1.0	2.4	11.4
	2	3	50 (I.V.)	50	7.6	1.5	6.1	12.2
6	2	2	20 (I.V.)	20	1.2	0.6	0.6	3.0
	1	2	50 (I.P.)	50	2.7	0.6	2.1	4.2
	1	1	25 (I.P.)	25	1.0	0.3	0.7	2.8

From the data in this report it appears that desoxycorticosterone may be metabolized to a 17-ketosteroid or other ketosteroid. Previous studies on the metabolic fate of desoxycorticosterone have indicated that this steroid may be converted to pregnanediol-3(α), 20(α) in humans and chimpanzees (Horwitt, Dorfman, Shipley and Fish, 1944) and in rabbits (Westphal, 1942; Hoffman, Kazmin and Browne, 1943). Other studies have indicated that the administration of desoxycorticosterone to an adrenalectomized monkey (Dorfman, Horwitt, Shipley and Abbott, 1944) may give rise to substances possessing adrenal cortical activity. These later metabolites have not been chemically identified.

The demonstration that the administration of whole adrenal cortical extracts give rise to 17-ketosteroids is in keeping with the

substantial body of evidence indicating the production of 17-ketosteroid precursors by the adrenal gland. These experiments, however, give no clue to the specific adrenal cortical steroids involved in this conversion.

Three 17-ketosteroids have been isolated from adrenal cortical extracts. These are androstanediol-3(β), 11(β)-one-17, adrenosterone, and Δ^4-androstenedione-3,17. The latter compound has been shown to be converted to androsterone in humans (Dorfman and Hamilton, 1940). The former two 17-ketosteroids may also contribute to 17-ketosteroid titer of the urine. It should be mentioned that it is not certain whether these three 17-ketosteroids are present in the gland as such or are formed during the process of isolation (Reichstein and Shoppee, 1943.)

The C_{21} adrenal cortical steroids may also be converted to 17-ketosteroids. Some of these 17-ketosteroids metabolites would presumably have ketone or hydroxy groups at C_{11} while others would not, depending on the specific precursors.

SUMMARY

Excretion of 17-ketosteroids and androgens was studied in five male and four female intact monkeys, and after gonadectomy or adrenalectomy, or gonadectomy-adrenalectomy. After gonadectomy the urinary excretion of 17-ketosteroids usually (but not invariably) increased during the first postoperative week. This was followed by a secondary decrease in 17-ketosteroids either to the preoperative level (in the female) or to a value below the preoperative level (in the male). The excretion of androgens decreased after castration in male monkeys. Ovariectomy resulted in a decreased androgen excretion in one female monkey and no change in a second female.

Even after the removal of the gonads and adrenals, both the male and female monkeys continued to excrete about one-third the preoperative level of 17-ketosteroids and androgens. Since all the adrenalectomized-gonadectomized animals died of adrenal insufficiency, and since no adrenal tissue could be detected at autopsy, the possibility exists that there may be an extra-gonadal, extra-adrenal source of 17-ketosteroids and androgens.

The administration of desoxycorticosterone to an ovariectomized chimpanzee, to an ovariectomized-adrenalectomized monkey and to patients suffering from Addison's disease caused distinct increases in 17-ketosteroid excretion. In the case of the Addison's disease patients it is difficult to discern whether this increased excretion of 17-ketosteroids was due to the general improvement of the physical status of the patient and the secondary increased production of 17-ketosteroid precursors by the gonads, or whether a real conversion took place. The former explanation seems more plausible. The experi-

ments on the ovariectomized chimpanzee and on the ovariectomized-adrenalectomized monkey indicated conversion of 3.2% to 13.8% for desoxycorticosterone to 17-ketosteroids.

The administration of adrenal cortical extracts to ovariectomized-adrenalectomized monkeys and to Addison's disease patients also gave rise to an increase in 17-ketosteroids, ranged from 2.8% to 12.2%. The data from the experiments in humans are difficult to evaluate for the reason previously stated.

The material represented by the term 17-ketosteroid would also include among others 3- and 20-ketosteroids.

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POTENTIATING EFFECTS OF ADRENAL CORTICAL EXTRACT ON INSULIN-INDUCED ABNORMALITIES OF CHICK DEVELOPMENT

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IT HAS BEEN SHOWN in earlier reports that the injection of insulin into chicken eggs leads to the appearance of embryo malformations. The kind of abnormality which insulin induces varies with the developmental stage during which embryos are exposed to the hormone. The principal effect of such treatment before the beginning and during the first 48 hours of incubation is rumplessness (Landauer, 1945). At later stages, especially during the fourth and fifth days of development, insulin chiefly induces micromelia and defects of the beak (Landauer, 1947).

It seemed of interest to determine whether or not other hormones have similar teratogenic properties and also what their possible interaction with insulin might be. The experiments on which we wish to report here were concerned with epinephrine and adrenal cortical extract and with tests in which one of these adrenal hormones was, either at the same time or after an interval of 24 hours, combined with insulin treatment.

METHODS

The procedure of injection was the same as described earlier (Landauer, 1945). All eggs came from a flock of White Leghorn fowl. The insulin was Iletin (Insulin, Lilly) U-40, and two units of this were used throughout. The epinephrine was dissolved in saline. The adrenal cortex preparation, furnished by Dr. E. C. Kendall, contained the extract of 150 gms. of glands per cc. All observations were made on newly hatched chicks and embryos which had failed to hatch or had died during the last four days of incubation. The results are expressed in percentages and their standard errors.

RESULTS

One series of experiments was concerned with those early stages of development (0 and 24 hours of incubation) during which insulin produces a considerable incidence of rumplessness. The sporadic frequency of rumplessness in untreated material is about 1.5 percent.

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The injection of either 0.02 cc. or 0.05 cc. adrenal cortex extract did not change this incidence (Experiments V j and k, table 1), nor did other abnormalities occur in significant numbers. The hormone did, however, produce a very marked retardation of embryo growth. With injection of the larger amount of adrenal cortex extract none of the embryos completed development, and with the smaller dose

TABLE 1. THE EFFECT OF INSULIN, ADRENAL CORTEX EXTRACT AND COMBINATIONS OF THESE HORMONES ON CHICK DEVELOPMENT AFTER INJECTION AT 0 OR 24 HOURS OF DEVELOPMENT

Experiment No.	Treatment	Survivors of 17th day	Rumpless %
IVa	Insulin—0 hours	100	25.0 ± 4.3
Va	Insulin—0 hours	78	34.6 ± 5.3
Vb	Insulin + 0.02 cc. saline—0 hours	56	37.5 ± 6.4
Vc	Insulin + 0.02 cc. saline—24 hours	42	40.5 ± 7.8
IVb	Saline 0.01 cc.—0 hours; insulin—24 hours	69	40.6 ± 5.9
IVc	Insulin—24 hours	108	17.6 ± 3.6
IVd	Adrenal cortex extract 0.01 cc. + insulin—0 hours	43	18.6 ± 6.1
Vd	Adrenal cortex extract 0.01 cc. + insulin—0 hours	51	31.4 ± 6.5
Ve	Adrenal cortex extract 0.02 cc. + insulin—0 hours	110	31.8 ± 4.5
IVe	Adrenal cortex extract 0.01 cc.—0 hours insulin—24 hours	62	25.8 ± 5.7
Vf	Adrenal cortex extract 0.02 cc.—0 hours insulin—24 hours	26	23.1 ± 7.7
IVf	Adrenal cortex extract 0.01 cc. + insulin—24 hours	65	26.2 ± 5.4
Vg	Adrenal cortex extract 0.02 cc. + insulin—24 hours	30	36.7 ± 8.8
IVg	Insulin 0 hours, 0.01 cc. adrenal cortex extract—24 hours	72	37.5 ± 5.8
Vh	Insulin 0 hours, 0.01 cc. adrenal cortex extract—24 hours	61	39.8 ± 6.3
Vi	Insulin 0 hours, 0.02 cc. adrenal cortex extract—24 hours	36	22.2 ± 7.0
Vg	Adrenal cortex extract 0.02 cc.—0 hours	42	2.4 ± 2.4
Vk	Adrenal cortex extract 0.05 cc.—0 hours	54	1.9 ± 1.9

embryo mortality was still very high during the last few days before hatching.

In the experiments which were to test a possible interaction of insulin and adrenal cortex extract on chick development, the procedure of injection was varied. In some experiments adrenal cortex extract (0.01 or 0.02 cc.) and insulin (2 units) were injected simultaneously before incubation or at 24 hours of development. In other tests one of the two hormones was injected at 0 hours, the other at 24 hours. The results are given in table 1. In none of these experiments did the incidence of rumplessness differ significantly from that produced by insulin alone.

Epinephrine, in amounts of 60 γ per egg, did not interfere with

normal development and growth, nor did it appear to modify the effect of insulin when the two hormones were injected simultaneously or within 24 hours of each other (table 2).

Adrenal cortex extract, injected in amounts of 0.02 cc. or 0.04 cc. at 120 hours of incubation (Experiments Ie and IIe, table 3), retarded growth considerably, but did not cause an increased incidence of any kind of developmental defect. When, however, insulin and adrenal cortex extract were injected simultaneously at 120 hours of development the incidence of micromelia and beak abnormalities rose strikingly above that caused by insulin alone. This is shown by a comparison of the results of experiment Ic with Ia and b and IIc with IIa and b. The differences are in each case statistically highly significant. When injection of insulin at 120 hours was followed by treatment

TABLE 2. THE EFFECT OF INSULIN, EPINEPHRINE AND COMBINATIONS OF THE TWO HORMONES ON CHICK DEVELOPMENT

Experiment No.	Treatment	Survivors of 17th day	Rumpless %
VIIa	Insulin—0 hours	89	36.0 ± 5.1
VIIb	Insulin + 0.05 cc. saline—0 hours	33	57.6 ± 8.6
VIIc	Insulin + epinephrine 60γ in 0.05 cc. saline—0 hours	72	44.4 ± 5.9
VIId	Insulin—0 hours; epinephrine 60γ in 0.05 cc. saline—24 hours	91	42.9 ± 5.2
VIIe	Epinephrine 60γ in 0.05 cc. saline—0 hours	82	2.4 ± 1.7

with adrenal cortex extract at 144 hours, potentiation of the insulin effect was less extreme than after simultaneous administration at 120 hours, but still significant. The same was true for the reverse experiment, i.e. injection of adrenal cortex extract (0.01 or 0.02 cc.) at 120 hours and insulin at 144 hours of incubation.

As in the earlier developmental stage, epinephrine had no effect on development of chicken embryos when 60 γ were injected into eggs at 96 hours of incubation. The combined administration of insulin and epinephrine at 96 hours or of insulin at 96 hours and epinephrine at 120 hours did not change the incidence of abnormalities which are induced by insulin alone (table 4).

DISCUSSION AND SUMMARY

Adrenal cortex extract, injected into eggs either before the beginning of incubation or at 120 hours of development delayed growth of chicken embryos very markedly. This retardation of growth did not lead to the occurrence of malformations, although the hormone was

TABLE 3. THE EFFECT OF INSULIN, ADRENAL CORTEX EXTRACT AND COMBINATIONS OF THE TWO HORMONES, INJECTED SIMULTANEOUSLY OR CONSECUTIVELY AT 120 AND 144 HOURS OF DEVELOPMENT, ON THE INCIDENCE OF MICROMELIA AND BEAK DEFECTS

Experiment No.	Treatment	Survivors of 17th day	Micromelia %	Beak abnormalities
Ia	Insulin—120 hours	45	60.0 ± 7.3	13.3 ± 5.1
Ib	Insulin + 0.04 cc. saline—120 hours	73	39.7 ± 5.8	13.7 ± 4.0
Ic	Insulin + 0.04 cc. adrenal cortex extract—120 hours	74	100 ± 0.4	41.9 ± 5.7
Id	Insulin—120 hours; 0.04 cc. adrenal cortex extract—144 hours	85	72.9 ± 4.8	9.4 ± 3.2
Ie	Adrenal cortex extract 0.04 cc.—120 hours	121	0	0
IIa	Insulin—120 hours	37	48.6 ± 8.3	8.1 ± 4.8
IIb	Insulin + 0.02 cc. saline—120 hours	67	28.4 ± 5.5	7.5 ± 3.2
IIc	Insulin + 0.02 cc. adrenal cortex extract—120 hours	68	92.6 ± 3.1	47.1 ± 6.0
IId	Insulin—120 hours; 0.02 cc. adrenal cortex extract—144 hours	107	75.7 ± 4.1	29.9 ± 4.4
IIe	Adrenal cortex extract 0.02 c.—120 hours	118	0	1.7 ± 1.2
IIIa	Insulin—144 hours	296	53.0 ± 2.9	7.9 ± 1.6
IIIb	Adrenal cortex extract 0.02 cc.—120 hours; insulin—144 hours	216	88.9 ± 2.1	33.8 ± 3.2
IIIc	Adrenal cortex extract 0.01 c.—120 hours; insulin—144 hours	239	85.8 ± 2.3	21.3 ± 2.7

injected at developmental periods during which insulin produces a high incidence of morphological defects. It may be concluded that the insulin effect is not mediated through a retardation of growth *per se*.

The present experiments confirm our earlier observation that insulin, when injected into eggs at 120 hours of incubation, is responsible for a high incidence of micromelia and beak defects among the

TABLE 4. THE RESULTS OF INJECTION OF INSULIN, EPINEPHRINE OR A COMBINATION OF THE TWO HORMONES AT 96 HOURS OR 96 AND 120 HOURS OF DEVELOPMENT ON INCIDENCE OF MICROMELIA AND BEAK DEFECTS IN CHICKEN EMBRYOS

Experiment No.	Treatment	Survivors of 17th day	Micromelia %	Beak abnormalities %
VIa	Insulin + .05 cc. saline—96 hours	107	31.8 ± 4.5	10.3 ± 2.9
VIb	Insulin + 60γ epinephrine in .05 cc. saline—96 hours	88	40.9 ± 5.2	13.6 ± 3.6
VIc	Insulin—96 hours; epinephrine 60γ in .05 cc. saline—120 hours	99	38.4 ± 4.9	10.1 ± 3.0
VId	Epinephrine 60γ in .05 cc. saline—96 hours	119	0	0.8 ± 0.8

developing embryos. The frequency with which these abnormalities occur is greatly exaggerated by the simultaneous injection of adrenal cortex extract and insulin. Adrenal cortex extract has, however, no similar potentiating effect during early developmental stages when insulin treatment produces a high incidence of rumplessness. It is possible that insulin at the two periods interferes with different physiological processes and that only the one which is insulin-sensitive during the fourth and fifth days is of such a nature that it can be potentiated by adrenal cortex extract. It is clear, in any event, that in early embryonic stages and in regard to the genesis of morphological abnormalities adrenal cortex extract and insulin do not have such antagonistic effects as have been reported for the late embryonic and post-embryonic periods when the endocrine system of the organism is functioning.

Epinephrine appeared to have no effect on development of the chicken embryo, nor did it alter the nature or incidence of insulin-induced abnormalities when it was injected in combination with insulin.

The injection of saline in combination with insulin, and in an amount equal to or larger than the volume of adrenal cortex extract which was used, did not alter the effects of insulin in a consistent or significant manner.

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EFFECTS OF VITAMIN A DEFICIENCY ON THYROID FUNCTION STUDIED WITH RADIOACTIVE IODINE[1,2]

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THERE HAVE BEEN a number of investigations bearing on the effects of vitamin A deficiency on the thyroid gland of the rat following the original observation of McCarrison (1914). This author (1931) noted hypertrophy of the gland and abnormal distension of the follicles in vitamin A deficiency. Spence (1932) reported similar results. Mitzkewitsch (1934) and Uotila (1938) found that in avitaminosis A the follicles of the gland were filled with colloid and that the epithelial cells were flattened. This was interpreted as indicative of hypofunction. De Ruyter (1934) found that the thyroid became atrophied with epithelial degeneration in severe avitaminosis A. Coplan and Sampson (1935) reported that vitamin A deficiency produced definite hypertrophy of the thyroid gland in the female rat but consistent atrophy in the male.

Opposed to these findings is the work of Drennan, Malcolm and Cox (1931), Sampson and Korenchevsky (1932), Sure (1938), and Remington, Harris and Smith (1943). These authors found that avitaminosis A did not affect the thyroid gland, the criterion used being the weight of the gland.

Still another picture was presented by Schulze and Hundhausen (1939) who reported that avitaminosis A gave a "tendency to activation." Their conclusions were based on histological examination of the thyroid.

An allied effect, which has also been studied, is the action of excess vitamin A on the thyroid gland. The consensus of many workers, who have reported on the weights and histologies of thyroid glands when excess vitamin A was administered, is that a high A diet causes a decrease in the weight of the gland, diminution in amount of colloid, and epithelial hyperplasia (Drill, 1943). A discussion of the effect of

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[2] The data are from a thesis presented by Mortimer B. Lipsett to the Graduate School of the University of Southern California in partial fulfillment of the requirements for the degree of Master of Science.

excess vitamin A on hyperthyroidism has recently been presented by Simkins (1947).

The lack of agreement concerning the effects of vitamin A deficiency on thyroid function prompted this investigation. The use of I^{131} has made it possible to measure the physiological activity of the gland in severe avitaminosis A.

METHODS

The animals used were of mixed sexes of the stock colony of the University of Southern California strain. The dietary regime was such as to make the animals suitable for use in the biological assay for vitamin A. The parents were maintained for at least 2 months previous to breeding and for 14 days after birth of the litter on Sherman diet B (Sherman and Campbell, 1924) without the addition of added lettuce or meat. Litters were reduced to 7 at 3 days of age, and on the fourteenth day, mothers and litters were placed on a vitamin A-low diet. This diet is the same as the USP XII vitamin A-deficient diet, except that commercial casein is used in place of alcohol-extracted casein. The animals were weaned at 21 days and one group of 30 animals was placed on the USP XII vitamin A-deficient diet. The potassium iodide was omitted from both of the above diets so that the iodine content of the diet was 16–17 μgm./kgm. A second group of control animals was treated the same way but supplemented with 8 I. U. of vitamin A daily per os. A third group of 8 animals was available at this time. These animals had been on a similar diet containing about 20 mgm. of carotene per kgm. of diet.

The vitamin A deficient animals were maintained for 25 days on the deficient diet. Of these 30 animals, 4 died and the others showed xerophthalmia of varying degree. The controls and the high vitamin A animals were used when they had reached the same weight as the deficient animals.

The animals were injected intraperitoneally with a tracer dose of about 50 microcuries of I^{131} in isotonic saline. After varying periods of time the animals were chloroformed and weighed; the thyroids were carefully excised, trimmed, and weighed to the nearest tenth of a milligram. The glands were then placed in 2 ml. of 2 N NaOH and hydrolyzed in a boiling water bath for 15 hours. After hydrolysis the contents were made up to 25 ml., and suitable aliquots taken for determination of each iodine-containing fraction.

The separation of the iodine fractions of the thyroid follows closely that described by Perlman, Morton and Chaikoff (1941) with only minor modifications. For determination of total iodine, diiodotyrosine iodine, and thyroxine iodine, aliquots were digested and distilled according to the procedure of Taurog and Chaikoff (1946). The distillate was made up to volume, an aliquot dried and read in the Geiger-Muller counter.

CHECKS OF THE METHOD

To determine the reliability of the methods used, the following tests were made:

(1) Samples of diiodotyrosine containing I^{131} prepared by the exchange reaction of Miller et al. (1944) were digested and distilled. Recovery was $92 \pm 4\%$.

(2) I^{131} and I^{127} were added to desiccated thyroid and carried through the

hydrolysis and separation. The I^{131} was recovered quantitatively as inorganic iodine.

(3) Radioactive diiodotyrosine was added to desiccated thyroid and carried through the hydrolysis and separation. Amounts varying from 4 to 7% were found in the inorganic fraction, however none appeared in the butyl alcohol layer.

(4) Radioactive thyroxine prepared by Mr. Earl Frieden of this laboratory, was added to desiccated thyroid and carried through the hydrolysis and separation. A similar loss of iodine occurred, an average of 5% appearing as inorganic iodine. There was no radioactivity in the diiodotyrosine fraction.

It can be seen that the results reported tend to give a slightly higher inorganic iodine fraction due to the breakdown of thyroxine and diiodotyrosine. Because of the correction value which had to be applied whenever distillation was used, the values are uncertain to about $\pm 5\%$ of the reported value.

RESULTS AND DISCUSSION

Weights of the thyroid glands. As can be seen from Table 1 the, thyroid weights in the vitamin A-deficient animals, when expressed as a fraction of the body weight, are significantly higher than the controls. This, of course, only confirms what has previously been reported in the literature. However, the sex difference which has been reported by Coplan and Sampson (1935) was not observed in this work.

TABLE 1. THYROID WEIGHTS OF NORMAL AND VITAMIN A DEFICIENT RATS

Group	Sex	Number of rats	Average rat weight	Average thyroid weight[1]
			gm.	mg./100 gm. body weight
Control	M	6	86.0	11.2 ± 0.53
Control	F	7	75.9	11.9 ± 0.45
Vitamin A deficient	M	14	71.1	22.5 ± 1.4
Vitamin A deficient	F	12	68.1	20.5 ± 1.8

[1] Including the standard error of the mean.

Histology. The thyroid glands of two control and two vitamin A-deficient animals of each sex were examined histologically. The glands from the control animals were essentially normal; although, by comparison with other rats in the stock colony, the follicles seemed slightly enlarged. This was probably due to the relatively low iodine diet used. In the rats fed a vitamin A-free diet, some areas of the gland presented the typical picture of colloid goiter, while other areas showed degenerative changes. In the goitrous parts of the gland, the follicles were distended and the epithelial cells flattened. In the few glands studied there was no noticeable sex difference.

Iodine uptake and distribution. As the injected iodine in these experiments was present in tracer amounts, it served to label the animals' circulating iodine. Thus the distribution of the I^{131} reflects the movement of the circulating iodine.

The total amount of I^{131} taken up by the thyroid gland at various intervals after injection is shown in Table 2. It can be seen from these results that there was no difference in the total uptake of I^{131} per

TABLE 2. THE DISTRIBUTION OF RADIOACTIVE IODINE IN THE THYROID OF RATS

Group	Hours after I^{131} administration	Number of animals[1]	Average weights		Percent of administered I^{131} recovered in thyroid		Percent of I^{131} present in thyroid		
			Animal gm.	thyroid mg.	Total determined	Sum of 3 fractions determined	As thyroxine	As diiodotyrosine	As inorganic iodine
Control	4	3	—	—	15.9	16.2	12.5	74.9	13.5
	18	4	80	9.3	14.2	—	19.5	—	—
	24	3	—	—	23.8	22.2	20.5	64.0	9.7
	36	5	83	9.3	15.1	—	24.4	—	—
	48	4	—	—	17.6	15.6	23.6	55.9	9.3
	72	4	79	9.2	15.5	—	23.3	—	—
Vitamin A-deficient	4	4	59	9.7	17.8	16.8	5.3	76.7	12.2
	8	4	72	14.2	21.2	21.2	4.1	81.3	17.2
	24	5	68	14.7	17.0	17.0	9.8	63.4	25.7
	48	6	68	13.6	22.0	21.2	18.3	52.9	24.8
	96	7	76	19.1	12.3	11.6	12.7	58.9	21.7
High carotene	4	2	—	—	17.7	—	11.7	—	11.8
	24	3	—	—	20.1	—	24.1	—	11.4
	48	3	—	—	14.2	—	29.8	—	8.1

[1] Due to the small numbers of animals in each group, statistical analysis of each average is not included. However, the individual values represented in the last three columns showed only minor deviations from the average values reported for each group.

whole gland in the 3 groups. However, the uptake per mgm. of thyroid tissue is appreciably lower in the vitamin A-deficient animals. It is possible that the goitrous areas of the glands of the vitamin A-free rats picked up iodine faster than normal and so compensated for the areas in which there were degenerative changes.

Conversion of inorganic iodine to organic iodine. Figure 1 is the graph of the values tabulated in Table 2. The curve which was obtained for inorganic iodine in the control animals is very similar to that obtained by other investigators. The inorganic iodine in the thyroid reached a maximum of about 15% of the total thyroid iodine within 4 hours after injection and then rapidly dropped to an almost constant value of about 9% of the thyroid iodine. The vitamin A-deficient animals, on the other hand, showed a rising inorganic iodine fraction which reached its maximum of 25% of the thyroid iodine at about the same time at which the iodine in the thyroid was maximal. There was then a very gradual fall of the inorganic fraction instead of the rapid drop found in the controls. These differences are very striking when it is realized that about the same percentages of injected I^{131} were picked up by the thyroid gland in each group of ani-

mals. These results are most reasonably interpreted by assuming that the thyroid gland in severe avitaminosis A is unable to convert inorganic iodine to organic iodine as rapidly as the normal gland.

The animals which were on a high vitamin A diet showed the same uptake and subsequent rapid fall of inorganic iodine as was obtained in the controls. The variations from the values obtained with

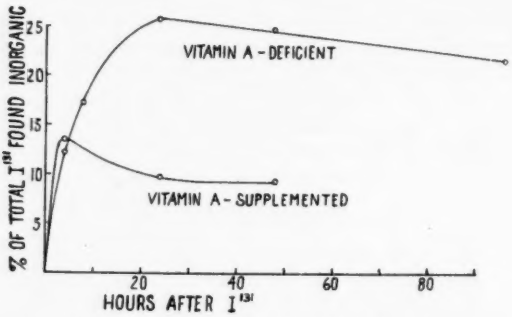

FIG. 1. Inorganic radioiodine content of thyroid glands from normal and vitamin A-deficient rats.

the control animals were not significant, but the values for inorganic iodine tended to be lower than in the controls.

Rate of formation of thyroxine. In the control animals, the I^{131} of the thyroxine fraction in the thyroid reached its maximum at about twenty-four hours after injection. There was subsequently a slow fall, a value of 23% of the total thyroid iodine being attained at 72 hours. This is shown in Figure 2.

With the group of vitamin A-deficient animals, the values for per cent of total thyroid iodine found as thyroxine iodine were lower at every point than the corresponding values for the control

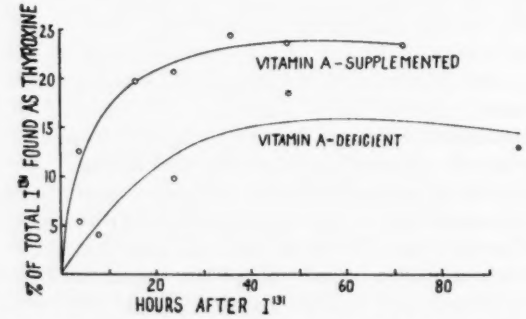

FIG. 2. Radiothyroxine content of the thyroid glands from normal and vitamin A-deficient rats.

animals. The differences here is not as striking as the difference in the per cents of inorganic iodine. However, there was a significantly lower rate of formation of thyroxine in the vitamin A-free rats and a decreased final level at 96 hours. It is impossible to tell whether the lack of vitamin A hindered the conversion of inorganic iodine to diiodotyrosine, of diiodotyrosine to thyroxine, or both.

With the group of high vitamin A animals, the values for the thyroxine fraction were higher than in the control animals. However, owing to the limited number of animals used, it cannot be stated that there is a positive effect of vitamin A on the formation of thyroxine. The values obtained only tend to suggest this possibility.

SUMMARY

The functioning of the thyroid gland of the rat was investigated in severe avitaminosis A, using radioactive iodine as a tracer. The following results were obtained:

1.) In vitamin A deficiency, the thyroid glands of the rat were relatively heavier than in control animals.

2.) The histological picture of the thyroid glands of vitamin A-deficient animals showed degenerative changes and distended follicles present within the same gland.

3.) Despite the increase in size of the thyroid glands of the vitamin A deficient rats, the total I^{131} uptake was the same as in the control animals.

4.) In vitamin A deficiency the percentage of inorganic I^{131} in the thyroid gland reached higher than normal values and decreased more slowly than in the controls.

5.) Vitamin A deficiency decreased the rate of formation of thyroxine. These results indicate that iodine metabolism is abnormal in the vitamin A-deficient rat.

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OVARIAN INFLUENCE ON THE RESPONSE OF THE ANTERIOR PITUITARY TO ESTROGENS

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THERE ARE TWO THEORIES of the action of sex hormones on the gonadotrophic function of the hypophysis. One theory is that sex hormones (estrogens or androgens) inhibit or suppress the gonadotrophic function of the hypophysis; the other is that the sex hormones stimulate the hypophysis to elaborate and release increased quantities of gonadotrophic hormone.

Review of the evidence supporting these concepts indicates that one is based on the results of long-term experiments and the other on interpretation of short-term experiments. Long-term experiments are those in which the rats, although immature initially, reached the normal age of maturity during the course of treatment. Furthermore, since the estrous cycle of the rat is 4 or 5 days in length and the gestation period is 22 days, many of the long-term experiments exceeded these normal reproductive processes in duration. The duration of short-term experiments more closely approximates the length of the sexual cycle.

Long-term experiments. The evidence for decreased gonadotrophic potency and function in rats as a result of estrogen treatment is found in long-term experiments, 10 to 60 days of treatment. The earlier experiments of this nature were summarized by Moore and Price (1932). Interpretation of findings after 17 to 20 days of treatment with estrogens or androgens led them to conclude that the sex hormones exerted a depressing effect upon the function of the hypophysis. Leonard, Meyer, and Hisaw (1931) treated immature female rats, (22 to 39 days of age) for periods of 20 to 66 days and found a decrease in the number of ripe follicles and corpora lutea in the ovaries of rats treated beyond 60 days. The pituitaries were increased in weight but decreased in potency. Meyer, Leonard, Hisaw and Martin (1932) treated castrate adult rats for periods of 13 to 37 days, and found that estrogen, 4 to 10 rat units per day, prevented the increase in pituitary potency that usually occurred after castration. Spencer, D'Amour and Gustavson (1932) gave daily injections of estrogen for 9 weeks to rats that were 3 to 4 weeks of age initially. At the end of the experi-

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ment the ovaries and the uteri of treated rats were smaller than those of the controls. The results were interpreted, as were those of Moore and Price, as indicating inhibition of pituitary function. Halpern and D'Amour (1936) treated mature rats for 4 or 8 weeks with 5 to 20 rat units of estrogen daily. They reported a progressive decrease in the size of the gonads, according to the dosage and duration of treatment. However, they agreed with Lane (1935) that there may have been initial stimulation followed by exhaustion of the gonadotrophic activity of the pituitary. Leonard (1937) treated castrate adult female rats with 10 rat units of estrogen daily for 10 days and found the pituitaries contained less gonadotrophic hormone. He also treated immature females for 10 to 22 days and concluded the pituitaries were depleted of luteinizing hormone.

Short-term experiments. Evidence of increased pituitary potency or the release of effective amounts of gonadotrophin is uniformly found in short-term estrogen experiments, 5 to 7 days. Burch and Cunningham (1930) found the pituitaries of spayed rats increased in potency after 6 days of estrogen treatment. Lane reported an increased number of ovarian follicles in immature rats and an increased pituitary potency after 5 days of estrogen. Mazer, Israel and Alpers (1936), Freudenberger and Clausen (1937), and Price and Ortiz (1944) obtained increases in ovarian weight as a result of injecting estrogen for 5 to 7 days. Hohlweg (1934) reported follicle stimulation and luteinization as a result of administering estrogen. These changes were also noted by Mazer, Israel and Alpers, and by Price and Ortiz, when the rats were 30 to 36 days of age at the initiation of treatment. Cole (1946) treated rats (21 to 23 days old) with estrogen and found that doses of 2 to 4 rat units daily for 6 to 10 days caused an increase in ovarian weight and the formation of ripe follicles or corpora lutea. The cytology of the pituitary after 5 to 7 days of estrogen administration has been interpreted as indicative of increased formation and release of gonadotrophic hormone, Severinghaus (1939), Baker and Everett (1944). These authors all believed that the initial effect of estrogen treatment was stimulation of the pituitary to increased elaboration and release of gonadotrophic hormone.

Since the estrous cycle of the rat is 4 or 5 days in length, it seemed that short-term estrogen experiments were more nearly physiologic in duration. This communication summarizes a series of experiments designed to reinvestigate the effect of 3 to 5 days of estrogen treatment on the ovary and on the gonadotrophic potency of the pituitary of the immature female rat. The earlier reports of a release of gonadotrophin and ovarian stimulation are confirmed. In addition, evidence is presented that the ovary accelerates this action of the estrogen on the pituitary.

MATERIALS AND METHODS

The rats used in this study were the Sprague-Dawley strain. Initial in-

jections were given on the 25th to 27th day of age. When spayed rats were employed, they were ovariectomized 24 to 48 hours before the initial injection.

Estrogens (estrone, estradiol benzoate, estradiol dipropionate, Westerfeld's lactone and stilbestrol) were given as a single subcutaneous injection. Doses ranged from 1.3 to 200 gamma in a total volume of 0.1 to 0.4 cc. of sesame oil and the duration of the experiment varied from 48 to 120 hours. At autopsy the ovaries and pituitaries were removed and weighed. The ovaries were fixed in Bouin's fluid for histological preparation and the pituitaries were saved for assay of gonadotrophic hormone content.

The pooled pituitaries of a test group (6 to 14 rats) were crushed into a smooth paste between two glass slides. The slides were separated and the film of pituitary substance was allowed to dry in room air and then stored in a desiccator unless it was to be assayed immediately. The wet or dried film was eluted from the slides with dilute alkali (1 drop N/10 NaOH in 50 cc. water), 2 cc. per pituitary. The assay rats were given this pituitary solution in doses of 0.5 cc. twice daily for 3 days, the total dose being equivalent to 1.5 or 2 pituitaries as designated. The rats were autopsied 72 to 76 hours after the initial injection. In order to avoid differences due to variations in sensitivity of different shipments of rats and to the environmental temperatures of different experiments, groups of control rats were used in as many experiments as possible.

EXPERIMENTAL

When estradiol dipropionate was given in doses of 2 to 20 gamma there was an increase in ovarian and hypophyseal weights in 96 hours (Experiments 1 and 2, table 1). The ovarian weight increase was more consistent when the dose of estrogen was 10 or 20 gamma and was still apparent after 120 hours (Experiment 3). The loss of pituitary gonadotrophic potency at 96 hours seemed equally great whether 2 or 20 gamma was administered. When the rats were ovariectomized before treatment with estradiol there was no appreciable loss of hormone from the pituitary (Experiments 4 and 5). There may have been a tendency to prevent the increase in pituitary potency which follows ovariectomy but the estrogen treatment, even a 200 gamma dose, did not prevent the potency from increasing over that of the normal controls (Experiment 6).

In experiments with estradiol benzoate, the increased weight of the ovaries and pituitaries was evident by 72 hours (Experiments 7, 8 and 9). The loss of pituitary potency was as great at 72 hours as at 96 hours. In the untreated castrate rats there was no evidence of an increase in pituitary potency over that of the untreated controls (Experiments 9 and 10). Estradiol benzoate did not cause any loss of gonadotrophic potency in the spayed animals and may have caused a slight increase in potency in experiments 9 and 11.

Estrone did not produce any increase in ovarian weights at doses of 6 to 10 gamma in experiments of 48, 72 and 96 hours duration in earlier experiments, data not shown. The dose was increased to 20 gamma and still no ovarian weight increase was obtained in 72 and 96

TABLE 1. OBSERVATIONS ON THE OVARIAN WEIGHT AND ON THE PITUITARY WEIGHT AND ITS GONADOTROPHIC HORMONE CONTENT IN NORMAL AND CASTRATED INFANTILE FEMALE RATS AFTER TREATMENT WITH ESTROGENS IN VARIED DOSES FOR 48 TO 120 HOURS

Expt. no.	No. rats	Treatment			Ovaries mg. av.	Pituitary mg. av.	Assay rats	
		Estrogen	Dose gamma	Time hours			No. rats	Ovaries mg. av.
1	10 Control		—	—	12.9	2.1	5	32.9
	8 Normal	Est. dip.	2	96	13.5	2.8	5	12.0
	8 Normal	Est. dip.	20	96	16.7	3.0	5	18.9
2	8 Control		—	—	14.4	2.7	5	43.8
	8 Normal	Est. dip.	2	96	23.5	3.3	5	12.5
	8 Normal	Est. dip.	20	96	23.5	3.5	5	13.8
3	8 Control		—	—	16.8	3.0	5	26.5
	8 Normal	Est. dip.	2	120	17.5	3.4	5	15.1
	8 Normal	Est. dip.	10	120	22.6	3.9	5	13.6
4	10 Normal	Est. dip.	2	96	17.9	3.3	4	17.0
	8 Castrate	Est. dip.	2	96	—	3.3	5	46.0
	8 Castrate		—	—	—	2.8	5	78.8
5	9 Control		—	—	—	2.6	5	39.3
	8 Castrate		—	—	—	2.6	5	68.5
	8 Castrate	Est. dip.	20	96	—	3.4	5	44.1
6	8 Control	—	—	—	—	2.6	5	24.8
	8 Castrate		—	—	—	2.9	5	57.1
	8 Castrate	Est. dip.	200	96	—	3.3	5	41.5
7	13 Control		—	—	15.3	2.6	8	32.5
	14 Normal	Est. ben.	1.3	48	14.9	2.9	8	26.2
	14 Normal	Est. ben.	1.3	72	20.7	3.0	8	20.1
8	16 Control		—	—	13.8	2.3	8	24.7
	16 Normal	Est. ben.	2.6	72	17.4	2.7	8	18.6
	16 Normal	Est. ben.	2.6	96	15.6	2.7	8	17.4
9	13 Control		—	—	16.3	2.4	8	33.2
	14 Normal	Est. ben.	1.3	72	22.6	3.2	8	21.5
	12 Castrate		—	—	—	3.0	8	33.7
	12 Castrate	Est. ben.	1.3	72	—	3.4	8	44.0
10	9 Control		—	—	—	2.0	6	48.5
	9 Castrate		—	—	—	2.5	6	45.4
	9 Castrate	Est. ben.	1.3	72	—	2.5	6	47.6
11	10 Control		—	—	12.6	2.3	6	38.6
	10 Normal	Est. ben.	1.3	96	16.3	2.8	6	25.5
	10 Castrate	Est. ben.	1.3	96	—	2.7	6	46.4
12	10 Control		—	—	14.7	2.7	5	48.2
	8 Normal	Estrone	20	72	15.3	3.1	5	44.3
	8 Normal	Estrone	20	96	13.7	2.6	5	24.9
13	8 Normal	Estrone	10	96	19.7	3.5	5	14.4
	8 Normal	Estrone	10	120	16.8	3.3	5	14.0
14	8 Control		—	—	—	2.7	5	72.1
	8 Castrate		—	—	—	3.0	5	64.6
	8 Castrate	Estrone	40	96	—	3.0	5	56.5
15	8 Control		—	—	—	2.3	5	35.1
	16 Castrate	Estrone	20	96	—	2.6	10	34.7

TABLE 1—(*continued*)

Expt. no.	No. rats	Treatment			Ovaries mg. av.	Pituitary mg. av.	Assay rats	
		Estrogen	Dose gamma	Time hours			No. rats	Ovaries mg. av.
16	9 Control		—	—	14.2	2.4	5	51.2
	8 Normal	Stilbestrol	20	96	18.4	3.2	5	27.9
	8 Castrate	Stilbestrol	20	96	—	3.1	5	57.6
17	8 Control		—	—	17.8	3.0	4	30.5*
	10 Normal	Lactone	20	96	16.6	3.6	4	29.7*
18	10 Normal	Lactone	20	96	15.2	3.4	4	38.4*
	10 Normal	Est. dip.	20	96	17.7	4.1	4	15.8*
19	10 Normal	Lactone	130	120	18.0	3.3	4	37.7*
	10 Normal	Estrone	200	120	28.6	4.4	4	17.0*
20	6 Control		—	—	17.7	3.6	3	47.9*
	6 Normal	Lactone	250	96	15.4	4.2	3	43.3*
21	10 Control		—	—	—	2.8	4	58.5*
	10 Castrate	Lactone	250	120	—	3.4	4	71.5*

Est. ben.—Estradiol Benzoate.
Est. dip.—Estradiol Dipropionate.
In Experiments 1 to 16 the assay rats were each given a dose equivalent to 1.5 donor pituitaries. In Experiments 17 to 21 the assay rats were given a dose equivalent to 2 donor pituitaries.*

hour experiments (Experiment 12). The loss of pituitary potency was evident at 96 hours but not at 72 hours. In experiment 13 the increase in ovarian weight was apparent at 96 and at 120 hours. Thus it appears that the release of gonadotrophin was 24 hours later with estrone than with estradiol benzoate, and the increase in ovarian weight was delayed correspondingly. Estrone in doses of 20 gamma and 40 gamma did not cause any marked loss of potency of pituitary of spayed rats (Experiments 14 and 15). In fact, none of the results with estrone were as decisive as those with the estradiol esters.

The slower action of estrone was interesting in relation to the hypothesis of Smith (1944), that estrone is metabolized to a substance, such as Westerfeld's lactone, which is more effective in releasing gonadotrophic hormone from the pituitary than is estrone. In the experiments of Smith and Smith (1944) stilbestrol was more effective than estrone in reducing the gonadotrophic potency of the male rat pituitary. They rationalized that since stilbestrol could not be converted to the lactone it must be active in its original form. In experiment 16, a 20 gamma dose of stilbestrol had an effect similar to that of estradiol, in that ovarian and pituitary weights were increased after 96 hours and the pituitary potency was reduced in normal but not spayed rats. Thus stilbestrol in equivalent dosage (20 gamma) was more effective or was effective earlier than estrone.

In experiments with the oxidation product of estrone, Wester-

feld's lactone acetate,[1] a dose of 20 gamma increased the pituitary weight but did not influence its potency or cause any increase in ovarian weight (Experiment 17). By contrast, a 20 gamma dose of estradiol diproprionate produced a marked decrease in the potency of the pituitary (Experiment 18). The lactone in doses of 130 gamma was less effective than estrone (200 gamma) in elevating pituitary and ovarian weights and lowering the pituitary potency (Experiment 19). Still higher doses of the lactone, 250 gamma, did not produce any demonstrable loss of hormone from the pituitary of either normal or castrate female rats (Experiments 20 and 21).

The most significant finding in these experiments with Westerfeld's lactone was that, even though the smallest dose (20 gamma) had sufficient estrogenic potency to induce vaginal patency and transitory cornification, the 250 gamma doses still did not have any effect on the gonadotrophic potency of the hypophysis. This substance, presumably the lactone, was thus the only estrogen used in this study which lacked this physiological action.

The experiments in this study probably should not be contrasted with those of the Smiths' since they used male rats and we used immature females. Furthermore, it has been pointed out by Smith (1944, 1946) that different preparations of the lactone vary in their estrogenic potencies and in their effects on the pituitary. Until a pure preparation of lactone is available, the physiologic effects may be expected to vary with the contaminants present in the preparations tested. Since Smith also admits difficulty in repeating her early experiments, the physiologic actions of the pure lactone, if any, can be established only when adequate amounts become available for study in several laboratories.

The consistent failure of any estrogen to reduce the potency of the hypophysis of castrate rats suggested that some other ovarian factor might be lacking. In a series of experiments with progesterone it was found that neither ovarian weight increases nor loss of pituitary potency occurred in intact rats given progesterone and estradiol benzoate, table 2 (Experiments 22, 23 and 24). This action of progesterone in blocking the release of gonadotrophin is in accord with the reports of Phillips (1937), Makepeace, Weinstein and Friedman (1937), Astwood and Fevold (1939), Selye (1939), and Smith and Smith (1944).

It was demonstrated by Moore and Price (1932) and verified by many others, that androgens and estrogens are similar in their ability to reduce the gonadotrophic potency of the pituitary in long-term experiments. It was of interest to determine whether testosterone would cause a release of hormone from the pituitary of the immature female rat in a manner comparable to that of estrogen. In experiments 25 to

[1] The author is indebted to Dr. Albert Segaloff, Alton Oshner Clinic for two samples of the lactone.

28, it was found that testosterone propionate in doses of 50 to 500 gamma had no consistent effect on either pituitary or ovarian weights nor was there any decrease in pituitary potency. Thus, within the limitations of these experiments as to dose and duration, testosterone did not have an action similar to that of estrogen.

In some circumstances, it has been shown that testosterone has an action similar to that of progesterone (Greene & Burrill, 1939). In

TABLE 2. OBSERVATIONS ON THE OVARIAN WEIGHT AND ON THE PITUITARY WEIGHT AND ITS GONADOTROPHIC HORMONE CONTENT IN NORMAL AND CASTRATED INFANTILE FEMALE RATS AFTER TREATMENT WITH ESTROGENS OR ANDROGEN OR ESTROGEN COMBINED WITH PROGESTERONE OR TESTOSTERONE FOR 72 TO 120 HOURS

		Treatment				Pituitary mg. av.	Assay rats	
Expt. no.	No. rats	Steroid hormones	Dose gamma	Time hours	Ovaries mg. av.		No. rats	Ovaries mg. av.
22	12 Control		—	—	—	2.3	7	31.0
	11 Castrate	Est. ben.	1.3	96	—	2.9	7	29.3
		Prog.	1000					
23	14 Control		—	—	—	2.3	8	41.7
	10 Castrate	Est. ben.	1.3	96	—	2.8	6	36.1
	9 Castrate	Est. ben.	1.3	96	—	3.0	6	34.3
		Prog.	1000					
24	10 Control		—	—	14.3	2.5	6	34.5
	10 Normal	Est. ben.	1.3	72	19.0	2.9	6	22.0
	10 Normal	Est. ben.	1.3	72	14.6	2.7	6	40.2
		Prog.	1000					
25	8 Normal	T. p.	50	96	16.0	2.7	5	22.9
	8 Normal	T. p.	500	96	16.0	3.0	5	26.2
	8 Castrate	T. p.	500	96	—	3.0	5	66.6
26	8 Control		—	—	14.3	2.1	—	—
	8 Normal	Est. dip.	20	96	22.0	3.6	—	—
	8 Normal	T. p.	500	96	15.1	2.8	—	—
27	8 Control		—	—	15.9	2.6	5	28.0
	8 Normal	T. p.	400	120	14.2	2.7	5	26.0
28	8 Normal	T. p.	500	96	16.2	3.1	5	34.6
	8 Castrate	T. p.	500	96	—	3.1	5	77.2
29	8 Control		—	—	15.4	2.7	4	32.8*
	8 Normal	Est. dip.	20	96	21.4	3.2	4	15.6*
	8 Normal	Est. dip.	20	96	16.9	3.3	4	16.4*
		T. p.	500					
30	6 Normal	Estrone	100	120	23.8	3.6	3	21.7*
	6 Normal	Estrone	100	120	19.8	3.3	3	39.1*
		T. p.	1000					

Est. ben.—Estradiol Benzoate.
Prog.—Progesterone.
T.p.—Testosterone Propionate.
Est. dip.—Estradiol Dipropionate.
In Experiments 22 to 28 the assay rats were given a dose equivalent to 1.5 donor pituitaries.
In Experiments 29 and 30 the assay rats were given a dose equivalent to 2 donor pituitaries.*

experiment 29, 500 gamma of testosterone propionate was given together with 20 gamma of estradiol dipropionate. The ovarian weight gain induced by the estrogen was inhibited by the testosterone but

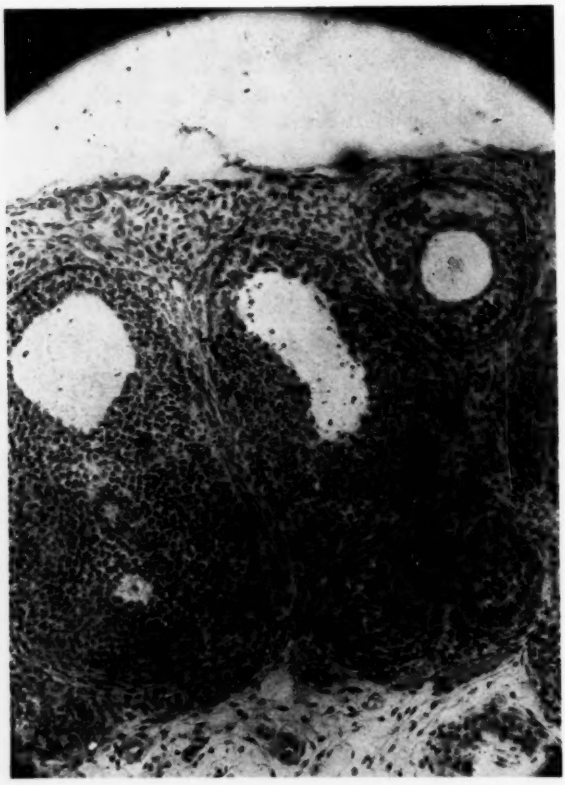

FIG. 1. Ovary from untreated control. Weight—16.0 mg. Note the closely approximated follicles with the narrow bands of thecal or interstitial tissue.

the pituitary weight gain and loss of potency were not altered. However, when 1 mg. of testosterone was given with 100 gamma of estrone the effects of estrone were inhibited (Experiment 30). Under these circumstances the action of testosterone is similar to that of progesterone.

The increase in ovarian weights due to estrogen treatment recorded in these experiments is similar to that noted by Lane (1935), Mazer, Israel and Alpers (1936), Freudenberger and Clausen (1937), Price and Ortiz (1944), and Cole (1946). Lane (1935), and Price and Ortiz (1944), noted an increase in follicle counts and an increased

number of larger follicles. Serial sections of the ovaries were not made in this study so information on follicular counts and follicle size was not obtained. There was however, a striking increase in the interstitial

FIG. 2. Ovary of immature female rat 96 hours after injection of 10 gamma estradiol dipropionate. Weight—26.3 mg. Note the broad bands of thecal interstitial tissue that have separated the follicles. The interstitial tissue has undergone a luteal type of transformation and is very vascular.

tissue (Figures 1 and 2). This increase in interstitial tissue is very similar to that present in the ovary after 48 hours treatment with human chorionic hormone. It seems probable that the increased vascularity and the epithelioid change in the interstitial tissue may account for at least part of the increase in ovarian weight in these experiments. These ovarian changes undoubtedly represent a response to the gonadotrophic hormones released from the pituitary, while the marked interstitial change suggests that the hormone may be primarily luteinizing (interstitial cell) stimulating in character.

DISCUSSION

The increase in pituitary weight induced by small doses of estrogen is similar to that reported by Baker and Everett (1944); the ovarian weights of their test animals were also increased. They interpret the pituitary cytology as indicative of an increased production of gonadotrophin hormone as a result of short-term treatment.

Lane (1935) stated that the initial effect of estrogen was to release follicle-stimulating hormone, and that the luteinizing hormone was released later. Fevold, Hisaw, and Greep (1936) concluded that estrogen caused a release of luteinizing hormone, since 3 days pretreatment with estrogen produced an augmented response to subsequent injections of follicle-stimulating hormone.[2] They were unable to demonstrate any such augmentation if estrogen was given for 8 days before the follicle-stimulating hormone was injected so they concluded that the luteinizing hormone of the pituitary had been depleted. Leonard (1937) obtained similar results in immature rats after 10 days of estrogen treatment prior to injecting gonadotrophic extracts.

The failure of estrogen to release the gonadotrophin from the pituitary of immature castrate female rats has not been reported previously. However, it seems similar to the observations in adult castrates. Burch and Cunningham (1930) injected 5 to 25 rat units daily for 6 days and reported an increased pituitary hormone content in estrogen treated castrates. Leonard (1937) did not succeed in reducing the potency of the adult castrate pituitary by estrogen in doses of 10 rat units daily for 10 days. Lauson, Heller and Severinghaus (1938) reported that 5 gamma of estradiol (60 rat units) daily for 20 days did not prevent the pituitary from increasing in potency after ovariectomy. Emery (1943) found that 100 gamma of estrone

[2] Unfortunately this augmentation experiment has never been confirmed. The author has failed repeatedly to achieve any effects similar to those reported by Fevold, Hisaw and Greep. Estrone or estradiol benzoate given 48 to 72 hours previously or simultaneously with crude pituitary extracts or purified preparations of F.S.H. has not produced any augmentation of ovarian weight or formation of corpora lutea. Smith (1945) reported difficulty in obtaining an F.S.H. preparation which would show augmentation with estrogen. Actually, the augmentation which she did obtain was opposite to that of Fevold, Hisaw and Greep, in that the estrogen was given after the F.S.H. rather than prior to it. Furthermore, the augmentation was obtained with the lactone rather than estrone. Frank and Berman (1941) published some findings which they interpreted as confirming Fevold, *et al.*, but their "a priori" assumption led them to 3 erroneous conclusions. They state that the release of the luetinizing factor "does not occur within the 96 hours of the bioassay, and it is not elicited by estrogenic injections unless gonadotrophic factor is added." These two conclusions are refuted by the present experiments. Their third conclusion that the luteinizing hormone is not released by the "dosage range of estrogen presumably produced by the animal's own growing follicle" is refuted in unpublished experiments. The pituitaries of immature rats given doses of F.S.H. or A.P.L., which stimulate the ovaries to produce enough estrogen to induce uterine enlargement and vaginal patency in 96 hours, have the same increase in weight and loss in gonadotrophic potency as that noted in the estrogen treated rats in the present study.

daily for as long as 50 days did not reduce the potency of the pituitary of spayed rats to that of the normal, even though he judged this dose to be "5 to 7 times that normally secreted by the intact ovaries." Meyer, Biddulph and Finerty (1946) noted that estradiol administered to castrate female rats for 10 days caused a marked increase in the gonadotrophic potency of the pituitary. Hellbaum and Greep (1946) demonstrated a decrease in luteinizing effect of the pituitary only after 30 to 45 days of estrogen injections.

The failure of estrogen to reduce the gonadotrophic hormone content of the pituitary of the spayed rat to that of the intact animal has given rise to a concept of ovarian utilization of gonadotrophin. Heller, Heller and Sevringhaus (1942), and Jungck, Heller and Nelson (1947) postulate that normally the ovaries metabolize the gonadotrophic hormones; whereas, in the spayed animal these hormones accumulate in blood stream and the pituitary potency also increases. The idea is of interest here primarily in suggesting that the ovary itself may be more effective than injected crystalline estrogens in the regulation of pituitary function.

The uniformity with which shorter periods of estrogen administration have failed to reduce the potency of the pituitary of spayed animals compared to that of the normal animal presents a number of possibilities for consideration.

1. Possibly the replacement doses of estrogen have been inadequate. In these experiments, low doses of estrogen (1.3 to 2 gamma) were effective in intact rats; whereas, doses of 20 to 200 gamma were ineffective in castrates. It is highly improbable that the infantile ovaries in the intact rats were producing enough estrogen to make this difference.

2. Possibly there is some other factor in the ovary. Progesterone is not this factor since it counteracts the effect of estrogen on the pituitary. The work of Smith and Smith indicates that progesterone depresses the rate of inactivation of estrone (oxidation to lactone) and thus reduces its pituitary stimulating activity. If their work is verified it may be assumed that the ovary increases the utilization or metabolism of estrogens and thus enhances their effectiveness.

3. Possibly estrogen has a direct effect on the ovary. Pencharz (1940) and Williams (1940) found that estrogen maintained the follicular structures in the ovary of hypophysectomized rats and increased the ovarian responsiveness to chorionic gonadotrophin. Williams (1944 and 1945) has continued his studies and concludes that the estrogen has a direct effect on the ovary which increases its sensitivity to injected gonadotrophic extracts. Although an increased sensitivity might explain the increase in ovarian weights in our experiments it does not explain how the presence of the ovary facilitates the release of gonadotrophin from the pituitary. It is possible that estrogen may stimulate the ovary to produce a substance which is

more effective on the pituitary than is estrogen itself. Further investigations are necessary to determine whether there is another substance in the ovary which facilitates the action of estrogen or whether the ovary influences the rate of utilization and metabolism of estrogen and thereby enhances its action on the pituitary.

The loss of pituitary potency of the intact female rat 72 to 96 hours after estrogen administration should be considered as a release of hormone under stimulation, rather than an inhibition or depression of function. The role of the ovary in this release mechanism must be investigated before all the intricate inter-relationships of the sex cycle can be explained. Long-term experiments in the rat, such as those of Moore and Price, have given rise to some generalizations which may not be applicable to the normal 4 or 5 day ovarian cycle. More emphasis should be given to experiments in which the duration and the dosage more nearly approximate the physiological norm for the species being investigated.

SUMMARY

When estrogen is given to the immature female rat there is an increase in ovarian and pituitary weight in 72 to 120 hours. The pituitary decreases in gonadotrophic hormone content in 72 to 96 hours. The loss of pituitary potency precedes the increase in ovarian weight, indicating that effective quantities of gonadotrophin are released into the blood stream. Small doses of estrogen, 1 to 2 gamma, are sufficient to produce these effects.

If the rats are spayed the day before the initiation of estrogen treatment, the pituitary does not decrease in potency in 96 hours even though the dose of estrogen is increased up to 200 gamma.

A preparation similar to Westerfeld's lactone was the only estrogen which failed to produce the effects described above in the intact infantile rat. Testosterone in doses up to 0.5 mg. did not affect ovarian weight or reduce the gonadotrophic potency of the pituitary. Progesterone or testosterone in doses of 1.0 mg. given simultaneously with the estrogens nullified their effect on ovarian weight and pituitary potency.

The ovary accelerates or augments the estrogen stimulation of the pituitary of the infantile female rat. Possible mechanisms whereby the ovary may participate in this action are discussed.

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NOTES AND COMMENTS

INFLUENCE OF THIOURACIL ON THE BASAL METABOLIC RATE AND ON MOLTING IN HENS[1]

In earlier papers (Benedict, Landauer and Fox, 1932; Perek and Sulman, 1945) the basal metabolic rate has been studied in molting and laying hens. The present authors found that the BMR averaged 400 to 500 cc. O_2 per kg/hr. in laying Leghorn hens in summer and winter. It increased to 600 to 700 cc. O_2 per kg/hr. during molting. Furthermore, it was found that administration of Lugol's solution or diethylstilbestrol failed to check or to accelerate molting.

Astwood, Bissell and Hughes (1944) studied the inhibition of the endocrine function of the chick thyroid. One tenth of one per cent of thiouracil in the food induced maximal thyroid enlargement. Thiouracil given as a 0.5 per cent mixture in the food retarded growth and development. Wattles, combs and spurs failed to develop, muscles were weak, joints hypermobile, and the chicks eventually became unable to stand. The body fat was increased. A fringe of wing feathers grew during the first few weeks but the down persisted and body feathers did not appear. The effect of thiouracil on the chick thyroid has been repeatedly studied and there is no doubt that thiouracil impairs the endocrine function of the gland, eventually inducing fattening.

Glazener and Jull (1946) reported on the effect of thiouracil administered orally to laying hens in a daily dosage of 0.45 g. The dosage was considered by the authors as the maximal tolerated dose, since higher doses were toxic. They found that molting was retarded in hens fed thiouracil. Mean body weights and egg production of the controls and the treated birds were approximately the same. Egg laying improved earlier, however, in the treated birds.

In this paper the effect of thiouracil on the BMR in molting hens was to be studied with the view to gaining an insight into the mechanism of molting and its relation to thyroid activity. It was obvious that the inhibitory effect of thiouracil should delay or prevent molting if the latter is initiated by the thyroid. Inability of thiouracil to influence molting would be a proof that molting is not initiated by the thyroid gland.

EXPERIMENTAL

The BMR was studied in 24 White Leghorn hens during the course of half a year, including the molting period. Every hen was tested 5 times monthly, and every test consisted of 2 to 3 readings. In addition, egg records were kept and inspection for molting as well as weighing was carried out once weekly. The method used was that described by Williams, Phelps and Burch (1941) for guinea pigs and adapted by Perek and Sulman (1945) for chickens. The hens were starved for 18 hours before every test, which lasted 2 to 3

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times 30 minutes. Molting was assessed by counting the feathers of the tail and wings. The hens were kept on a standard stock diet prepared as usual in this country for laying hens maintained in batteries. Eight hens received this diet throughout the period of investigation, whereas 4 groups of 4 hens each received the standard diet with the addition of 0.1 per cent, 0.2 per cent, 0.5 per cent and 1.0 per cent, respectively, of thiouracil, starting 2 months after preliminary testing of the hens' BMR in our batteries on August 1.

TABLE I. RELATIONSHIP BETWEEN THIOURACIL FEEDING (IN % OF FOOD AS FROM AUGUST 1), MOLT (AS DETERMINED BY COUNTING FEATHERS), EGG-LAYING (MONTHLY) AND BMR (CC. O_2 PER KG/H)

Group	Thio-uracil feeding	Hen No.	July	Normal food		August	Food containing Thio-uracil		September	Food containing Thio-uracil		October	Food containing Thio-uracil		November	Normal food	
			molt	eggs	BMR	molt	eggs	BMR	molt	eggs	BMR	molt	eggs	BMR	molt	eggs	BMR
I	0.1%	1	−	26	221	−	15	357	+	0	365	+	0	776	−	20	305
		2	−	24	270	−	12	280	−	21	296	±	6	261	−	21	286
		3	−	27	272	±	16	334	+	0	386	+	1	384	−	19	264
		4	−	24	387	−	21	479	−	18	401	±	13	424	−	22	390
II	0.2%	5	−	15	274	−	22	291	−	22	166	±	12	160	−	20	301
		6	−	19	375	−	19	311	+	7	395	+	0	361	−	18	305
		7	−	23	292	−	17	255	±	10	187	+	0	464	−	16	311
		8	−	25	205	−	16	243	+	1	315	+	0	212	−	17	283
III	0.5%	9	−	21	280	±	8	135	+	2	269	+	0	363	−	18	290
		10	−	23	220	+	5	314	+	0	274	+	0	224	−	19	287
		11	−	24	217	−	11	145	+	0	298	+	0	385	−	20	305
		12	−	25	280	±	9	279	+	0	270	+	0	338	−	16	315
IV	1%	13	−	22	354	−	20	131	+	5	210	+	0	202	−	18	292
		14	−	21	196	+	3	338	+	0	286	+	0	220	−	17	285
		15	−	9	312	+	0	341	+	0	221	+	0	165	−	10	294
		16	−	15	306	+	18	176	+	0	203	+	0	183	−	12	300
V	Control maintained on normal food	17	−	11	309	−	11	379	−	14	323	+	5	409	−	13	310
		18	−	28	323	−	25	303	−	18	236	+	0	494	±	10	305
		19	−	22	311	−	25	281	−	16	332	+	3	328	−	16	312
		20	−	20	379	−	24	363	−	23	300	+	6	407	−	13	308
		21	−	26	287	−	21	209	−	22	222	+	8	366	+	10	280
		22	−	25	235	−	23	361	±	9	470	+	1	474	−	15	291
		23	−	24	290	−	20	210	−	23	241	+	5	380	−	16	288
		24	−	12	314	−	13	381	−	16	351	+	0	401	±	10	326

N.B. BMR determinations and egg records were begun in June, the result did, however, not differ significantly from those obtained in July.

RESULTS

Table I shows the influence of thiouracil feeding on molting, egg-laying and the BMR. The experiments show that thiouracil induced molting rather than preventing it. Whereas the controls (group V) started molting only in October, the thiouracil-fed hens started as early as August (group IV). The molting effect was established earlier in the two groups receiving 0.5 per cent and 1 per cent thiouracil, respectively, than in groups I and II, receiving 0.1 per cent and 0.2 per cent thiouracil, in which molting began only in September. As in every natural molting period, egg-laying was diminished when molting began.

The depression of the BMR by thiouracil after one month's feeding was clearly detectable. In contradistinction to natural conditions where molting is associated with an elevated BMR, thiouracil-induced molting is accompanied by an unchanged or even a decreased BMR. This was very striking in group IV, receiving 1 per cent of thiouracil in its food. Moreover, these hens consumed less food than normal hens or than hens on a lower thiouracil ration. It should be noted that the hens in all 5 groups maintained their

initial weight (1500 to 1800 g.) during the whole period of thiouracil feeding, which lasted 3 months.

In conclusion it may be stated that the increased BMR naturally associciated with molting is not a *conditio sine qua non* for the initiation of this process, since we have been able to produce molting with unchanged or with decreased BMR.

DISCUSSION

The above experiments furnish us with a certain insight into the mechanism of molting, without solving its puzzle. It appears from these experiments that the thyroid gland does not necessarily stimulate molting, since depression of thyroid activity does not prevent this process. On the contrary, it has been shown that thiouracil initiates molting. It must be concluded, therefore, that the theory which assumes that molting is initiated by increased activity of the thyroid gland can no longer be maintained. We must assume that molting itself influences the thyroid gland in a secondary manner and stimulates its activity in order to make up for the loss of body heat resulting from the loss of feathers. A similar mechanism has been described by Landauer (1934, 1936, 1937) for the frizzle fowl. This conception confirms a suspicion mentioned as early as 1939 by van der Meulen, who advanced the theory that fluctuations of the secretion of thyrotropic and gonadotropic hormones induced molting and impaired egg-laying, the two mechanisms being quite independent of each other. In the light of our experience it seems reasonable to assume that the disturbance of the gonadotropic function is a primary one in molting, whereas the disturbance of the thyrotropic function is secondary. This possibility is hinted at by experiments of B. Zondek (1936, 1937), who showed that treatment of hens with excessive doses of estrogens leading to pituitary impairment, induces molting, without changing the structure of the thyroid gland or the BMR.

SUMMARY

The egg production, basal metabolic rate and molting of 24 White Leghorn hens were studied over a period of half a year. Thiouracil feeding depressed the BMR, without, however, preventing the molt. Its administration in the food initiated molting especially in a dosage of 0.5 to 1 per cent.

The bearing of these results on the mechanism of molting is discussed, since it appears that molting may occur in spite of a depression of the thyroid gland.

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THE ACTION OF DIETHYLSTILBESTROL AND SOME STEROIDS ON THE RESPIRATION OF RAT BRAIN HOMOGENATES[1]

SELYE (1941) has shown that certain steroids[2] are capable of producing a state of anesthesia in mammals when administered intraperitoneally or subcutaneously. Michaelis and Quastel (1941) suggested that the action of anesthetics is due to inhibition of carbohydrate oxidation produced by interrupting the usual reactions of some flavoprotein or other component of the tissue respiratory system which plays an intermediate role between flavoprotein and cytochrome oxidase.

Using conventional manometric methods, we have studied the effects of diethylstilbestrol and several steroids on the oxidation of glucose and succinate by rat cerebral cortex homogenates prepared in modified Krebs-Ringer phosphate (Elliott *et al.*, 1947) by means of a Potter (1936) homogenizer. Since the substances used were insoluble in water, the crystalline compounds were ground to a fine powder, suspended in Ringer's solution, and added from the sidearms of the Warburg vessels after a 60 minute control period. Readings were made every ten minutes for an additional 60 minute experimental period. In the usual case, 4 mg. of steroid was added to approximately 150 mg. of homogenized brain. Control preparations showed no fall in Q_{02} over the two hour period. The average wet weight Q_{02} of this preparation was 1.15.

The effects of the compounds studied on the oxidation of glucose and succinate are shown in table 1. With the exception of diethylstilbestrol, the ability of the steroids to inhibit respiration in the presence of glucose parallels the anesthetic potency as determined by Selye. In common with other anesthetics, the steroids have little effect upon the oxidation of succinate, diethylstilbestrol again excepted.

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TABLE 1. COMPOUNDS LISTED IN ORDER OF DESCENDING ANESTHETIC POTENCY. INHIBITION CALCULATED BY COMPARING Q_{O2} AT THE END OF THE EXPERIMENTAL PERIOD WITH THE AVERAGE Q_{O2} OF THE CONTROL PERIOD

	M.P. ° C.	A.R.U.† in mg.	Percent inhibition			
			0.2% glucose substrate	N	0.2 M succinate substrate	N
Desoxycorticosterone	134	1.0*	87	12	15	12
Progesterone	125	2.0	28	10	10	10
Testosterone	151	7.0	24	18	1	10
Stilbestrol	173	20.0	91	19	44	14
α-Estradiol	171	>20.0	14	9	3	9
Cholesterol	147	Inactive	14	10	0	10

† from Selye (1942).
* Selye's value for desoxycorticosterone acetate.
M.P. = melting point.
A.R.U. = anesthetic rat unit.
N. = number of vessels.

Studies are in progress to determine the site of action of steroids on the mechanisms of biological oxidation. Methylene blue (6×10^{-5}M) reverses the inhibition of the rat brain succinoxidase system produced by diethylstilbestrol and desoxycorticosterone. This finding supports the work of McShan and Meyer (1946) who found that diethylstilbestrol inhibition of rat liver and pituitary succinoxidase systems was effected through the cytochrome oxidase of the system. However, we have found that 6×10^{-5}M methylene blue does not reverse the inhibition of glucose oxidation by brain in any case. Therefore, it is unlikely that this action is produced by an effect on cytochrome oxidase. Since methylene blue can act as a carrier between dehydrogenases and oxygen (Grieg, 1946), its failure to reverse the inhibition of glucose oxidation indicates an action on the dehydrogenases of the cellular respiration system.

Thus, although there are certain parallels between the anesthetic action of steroids and their ability to inhibit brain tissue respiration, the site of action on the "main line of biological oxidation" is not the same for steroids as for other anesthetics which have been studied.

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